NUTRIENT AVAILABILITY FROM A HIGH-PROTEIN, HIGH-CALCIUM PASTA

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NOMENCLATURE

m-AIN	Marginal 10% protein American Institute of Nutrition rat diet modified by incorporating wheat starch rather than corn starch.
PA	Pasta Adequate: the 17% protein pasta diet, the amount of protein in the supplemented pasta.
PM	Pasta Marginal: the 10% protein pasta diet, where in the amount of protein in the pasta was diluted by the addition of wheat starch.

CHAPTER I

INTRODUCTION

For the average American, protein intake may exceed the minimum dietary requirements; but the frail elderly requirement is often unmet, leading to malnutrition. Further, some older adults need more calcium according to the Third National Health and Nutrition Examination Survey(1), which found examples of inadequate calcium intake. Unfortunately, increasing protein in diets of the frail elderly could make a marginal calcium status even worse, because dietary protein enhances the urinary excretion of calcium. The excretion of calcium occurs at both low and high protein intakes, but the urinary losses of calcium are greater at higher protein intakes. Estimates are that for each gram of metabolized protein, one milligram of calcium is lost in the urine(2). Our hunter-gatherer ancestors might have been healthy with a high protein and high calcium diet(3); however, in current diets there may be an imbalance of the protein to calcium. Therefore, when designing diets or food products to address one deficiency, such as protein, care should taken to avoid magnifying another deficiency, such as calcium.

Considering the nutritional needs and food intakes of these frail elderly, a pasta supplemented with protein and calcium has been developed at Oklahoma State University. A 100 g cooked serving of the pasta provides the amount of calcium in a glass of milk and 8 g of protein. A panel of active elderly persons has evaluated the pasta and rated it as very acceptable(4), but no tests were done for utilization of nutrients. The purpose of this

study, using rats as the experimental animal, was to determine the bioavailability of the protein in the supplemented pasta (by comparing it at marginal amounts to a standard diet) and to determine whether varying the protein amount in the diet of rats fed the pasta affected calcium utilization.

Objectives and Hypotheses

The three objectives, subdivided into two parts, of this research project are the following:

(1.1) To determine if a marginal diet, either from the modified American Institute of Nutrition rat 10% protein diet (m-AIN), or from a 10% protein pasta diet (PM) will equally affect the composition of the carcass and feces.

(1.2) To determine if reducing the pasta diet from 17% (PA) to 10% (PM) protein will affect the composition of the carcass and of the feces.

(2.1) To determine whether the protein from the m-AIN diet or the PM diet will be efficiently absorbed and utilized based on protein efficiency ratios and other protein measurements.

(2.2) To determine whether reducing the amount of protein from 17% (PA) to 10% (PM) from the pasta diets will interfere the absorbed and utilized based on protein efficiency ratios and other protein measurements.

(3.1) To determine if the marginal protein from either the m-AIN diet or the PM diet will interfere with the absorption and assimilation of dietary calcium.

(3.2) To determine whether the amount of pasta protein in either the PM diet or the PA diet will interfere with the absorption and assimilation of dietary calcium. To

accomplish objectives 1.1, 2.1, and 3.1, results from animals fed the marginal protein diets, the m-AIN and PM diets, will be compared. To accomplish objectives 1.2, 2.2, and 3.2, results from animals fed two levels of pasta protein, the 10% PM and the 17% PA diets, will be compared.

The three hypotheses, subdivided into two parts, of this research are the following:

H 1.1: There will be no differences between the results from the m-AIN diet and the results from the 10% protein pasta diet for selected carcass and fecal parameters (total feed intake, feed digestibility, total weight gain, animal final weight, carcass moisture, carcass dry matter, carcass fat, carcass energy, fecal weight, fecal fat, and fecal energy).

H 1.2: There will be no differences between the results from the marginal protein pasta diet and the results from the adequate protein pasta diet for selected carcass and fecal parameters (total feed intake, feed digestibility, total weight gain, animal final weight, carcass moisture, carcass dry matter, carcass fat, carcass energy, fecal weight, fecal fat, and fecal energy).

H 2.1: There will be no differences between the results from the m-AIN diet and the results from the 10% protein pasta diet for selected protein parameters (protein efficiency ratio, fecal protein, carcass protein, and serum albumin).

H 2.2: There will be no differences between the results from the marginal protein pasta diet and the results from the adequate protein pasta diet for selected protein parameters (protein efficiency ratio, fecal protein, carcass protein, and serum albumin).

H 3.1: There will be no differences between the results from the m-AIN diet and the results from the 10% protein pasta diet for selected calcium and bone parameters (fecal calcium, bone density, bone dry weight, bone ash weight, bone calcium, bone

phosphorus, bone breaking load, bone elongation at break, bone energy to peak, bone peak load, bone wall diameter, and bone wall thickness).

H 3.2: There will be no differences between the results from the marginal protein pasta diet and the results from the adequate protein pasta diet for selected calcium and bone parameters (fecal calcium, bone density, bone dry weight, bone ash weight, bone calcium, bone phosphorus, bone breaking load, bone elongation at break, bone energy to peak, bone peak load, bone wall diameter, and bone wall thickness).

Assumptions

In this experiment, the assumptions were:

1. Adequate amounts of all nutrients, except for protein in the marginal treatments, were offered to the animals.
2. Diets were prepared similarly according to accepted procedures.
3. Samples taken were representative of the whole.
4. Chemical analyses were precise and accurate.
5. Conditions in the animal feeding facility were sufficient for normal growth.
6. Cage arrangements were such that biases due to location were minimized.

Limitations

1. Only 27 rats (experimental units) were used in this study.
2. Only two protein amounts (10% or 17%) were provided by the diets.
3. The primary source of calcium was calcium carbonate for all diets.

Definitions

Breaking Load: The load (force) required to break a sample. After breaking the force decreases significantly, usually to zero.

Elongation at Break: The distance or displacement the sample has undergone at the instant when it breaks.

Peak Load: The maximum amount of force a sample undergoes during loading either before, at, or after breaking.

Protein Efficiency Ratio (PER): An index of protein quality determined by dividing the weight gain of a group of animals during 28 days of feeding by their protein intake during 28 days of feeding.

CHAPTER II

REVIEW OF LITERATURE: THE CALCIUM AND PROTEIN NEEDS OF THE ELDERLY

Introduction

Although older adults need many nutrients, a particular dilemma among the frail elderly is protein deficiency. However, increasing protein intake could exacerbate calcium deficiency. Clearly, when designing a food to increase one nutrient, care should be taken to avoid diminishing the absorption or use of another nutrient. This review addresses these concerns by examining protein and calcium recommendations, dietary calcium supplements, and considerations for designing foods for the elderly.

Protein Recommendations for the Elderly

The protein intake of many older adults in the United States is adequate, but is inadequate for others. In fact 2% to 15% of the healthy elderly have low protein intakes. However, for those who are institutionalized, the figures are worse; up to 33% consume insufficient protein(5). Nutritional problems exist especially for those with complicating factors, such as poor dentition or the need for nutrient dense foods. Table I lists these and other circumstances that discourage adequate protein intake. Thus, aged individuals with protein malnutrition may require "special palatable and protein-enriched meals(6)."

TABLE I

RISK FACTORS FOR MALNUTRITION
IN ELDERLY PEOPLE[a]

FOOD/FEEDING PROBLEMS
Difficulties procuring and preparing food
Lack of oral health
Poor sensory perceptions
Inadequate food intake
Inability to self-feed
Need for nutrient dense meals
Poverty
MEDICAL PROBLEMS
Infections
Polypharmacy
Anorexigenic drugs
Recent surgery
Chronic illness
Hyperactivity
PSYCHO-SOCIAL PROBLEMS
Recent loss of a spouse
Dementia
Depression
Institutionalization

[a]Compiled from several sources: (7,8,9,10,11).

Determinations of Needed Protein Intake

This section of the review compares several authors' protein intake recommendations. However, the authors use different measures and procedures for

basing their recommendations, so it was necessary to seek commonality. Therefore, to accomplish the comparisons, four steps are followed: first, the hypothesized protein needs or the protein reference values are compiled. Second, these recommended values are all converted to grams of protein, the unit measurement for the National Academy of Science's recommended dietary allowances (RDA). Third, the protein intake needs of a representative elderly man and woman, each weighing 68 kg, are calculated from their energy needs. Then, the grams of protein are transformed into a simple common metric, the percent RDA, for the benefit of comparison. Fourth, the different protein recommendations are compared to data from an actual food intake survey of those who are greater than 60 years old.

Recommended Intakes in Grams and Percentages for 68 kg Elderly Adults

Protein is an essential dietary component for tissue maintenance and repair, and enzyme production, even among the elderly. The usual protein intake of elderly persons 65 years and older is 75 to 80 g for men and 55 to 65 g for women(12). Based on the RDA, the recommended protein allowance is 0.80 g/kg, or 54.5 g/day, if weighing 68 kg, for those 51 years and older. However, this intake may be insufficient to meet their needs, if presented with the extraordinary protein requirements of disease.

In addition to the RDA, there are several other protein calculation strategies and recommendations for older adults. For these, protein recommendations involve factors beyond age and weight. Gender, disease, drug use, nutrient biochemical status, and physical activity should also be incorporated in the decision according to Blumberg(13). Another strategy for determining their intake is to consider protein as percent of caloric

intake. Twelve to fourteen percent of total kilocalories for those over 60 years old is recommended by Munro(14). Thus, with the estimated energy needs of 68 kg elderly persons being 1500 kilocalories for the woman and 1900 kilocalories for a man(15), their protein needs would be 45 - 54 g (83% to 99% of the RDA) for the woman, and 57 - 67 g (104% to 123% of the RDA) for the man. A protein requirement of 1.00 g/kg/d based on nitrogen equilibrium is recommended by Campbell, Crim, Dallal, Young, and Evans(16). They advocate protein intake, including a safety margin for the aged, of 1.0 to 1.25 g/kg/d. Thus, based on these estimates, the protein needs for the example elderly persons would range from 68 g - 85 g per day (125% to 156% of the RDA).

Besides assessing protein intake, evaluation of protein status uses other indices. One indicator, which when low may coincide with a depressed stress response(17), is the whole body protein turnover. The components of this index include: total amino acid flux (Q), body protein breakdown (B), amino acid intake (I), body protein synthesis (S), and amino acid catabolism (C). This equation expresses the relationship of these components:

$$Q=B+I=S+C \text{ (18).}$$

Pannemans, Halliday, and Westerterp(17) report that protein turnover is significantly greater ($p<0.0001$) with higher intakes of protein. That is, a diet consisting of 12% of its total energy as protein realizes lower protein turnover rates than a diet with 21% of its total energy as protein. Therefore, 21% is recommended as the intake adjustment to compensate for the increase in protein needs as total energy needs are declining within the elderly population. So, using the estimated kilocalorie needs of the subjects, based on 21% of energy as protein, their protein needs are 80 g (147% of the RDA) for the woman and 100 g (184% of the RDA) for the man.

In summary, after conversions are made and the various recommendations for protein are compared with each other and with the official RDA, the consensus seems to be that the protein intake needs for these elderly persons are 83% to 147% of the RDA for the woman and 104% to 184% RDA for the man.

Comparison of Recommended to Actual Protein Intakes

As the recommendations increase, a greater percentage of the elderly show inadequate intakes; thus more of them appear to be at risk of protein malnutrition. Protein reference values stated either as percent of kilocalories or in grams can be compared to protein intake data from the National Health and Nutrition Examination Survey(1) (NHANES III) (See Table II). During this survey, 24-hour food intake records were completed by 2,566 randomly selected U.S. civilian non-institutionalized people. The elderly people in the NHANES study consume greater than 12 - 14% of their calories as protein which according to Munro(14) is adequate. However, compared to 21% of kilocalories from protein, recommended by Pannemans et al.(17), most intakes are inadequate. The example elderly man and woman also show this pattern. Their actual protein intake is adequate as compared with the RDA, but deficient when compared to 21% of kilocalories or 1.0 to 1.25 g of protein per kilogram of body weight. If in fact NHANES III accurately reflects mean protein intakes of the elderly, the heightened protein needs for many elderly are largely unmet.

TABLE II

PROTEIN INTAKE BY RACE/ETHNICITY AND AGE (60+ yo): UNITED STATES, 1988 - 91[a]

	Total Population			Non-Hispanic White		
Age	Mean	Mean	Median	Mean	Mean	Median
Men	(% kcal)	(g)	(g)	(% kcal)	(g)	(g)
60-69y	16.4	84	78	16.3	85	79
70-79y	16.0	74	70	15.9	75	70
80+y	15.0	69	64	15.7	69	65
Women						
60-69y	16.6	64	60	16.4	64	60
70-79y	16.6	58	55	16.4	57	55
80+y	15.9	52	49	15.9	52	49
	Non-Hispanic Black			Mexican American		
Age	Mean	Mean	Median	Mean	Mean	Median
Men	(% kcal)	(g)	(g)	(% kcal)	(g)	(g)
60-69y	17.2	78	67	16.5	78	74
70-79y	17.1	63	60	17.8	73	64
80+y	18.9[b]	70[b]	56[b]	17.6	62[b]	60[b]
Women						
60-69y	16.2	56	51	17.5	56	53
70-79y	17.3	62	57	16.4	50	45
80+y	15.5	50	45[b]	13.9[b]	43[b]	41[b]

[a]Data based on 24-hr dietary recall, 1 day [b]Value does not meet standards of reliability or precision. Source: (1)

Health Effects of Protein Malnutrition in the Elderly

The severity of protein malnutrition among aged adults affects the response to disease and the duration of life. The mortality and malnutrition of 205 non-cancer subjects age 74 to 76 were studied by Cederholm, Jagren, and Hellstrom(19). They identify malnourished participants as those with at least three of the following indices below

reference amounts: weight index, triceps skinfold thickness, arm muscle circumference, serum albumin, or delayed cutaneous hypersensitivity reaction. The researchers report significant differences ($p<0.001$) in mortality rates among malnourished subjects versus well-nourished subjects. Most healthy elderly have adequate protein intakes. They are less active and through normal aging have a reduced body cell mass and lower basal metabolism and requires less. However, many elderly are unhealthy; and, thus, disease is a risk factor for protein-energy malnutrition (PEM) according to Schlienger, Pradignac, and Grunenberger(6). To recover from many diseases by repairing tissues and promoting the processes of the immune system, the body requires greater protein intake. When the increased protein needs of the frail aged go unmet, the results can include: muscle wasting, nutrient deficiencies, immunoincompetence secondary to higher energy expenditure, greater protein catabolism rates, and changes in sensory perceptions. Therefore, some of the elderly who suffer from diseases probably have deficient protein intake.

Calcium Recommendations for the Elderly

Many elderly in the U.S. have unmet calcium needs. They have difficulty meeting calcium needs for several reasons: inadequate intake, interference with absorption, and gastrointestinal changes. The calcium intakes of women over age 60 and men over age 80, according to NHANES III, are inadequate. Intakes less than 100% of the RDA are reported by this survey for these elderly (See Table III(1)). The inability of segments of the U.S. elderly population to meet their calcium requirements is exacerbated by dietary factors that reduce calcium availability. In addition, physiological changes in the elderly may enhance the need for dietary calcium. The purpose of this section of the review of

literature is to present information on various calcium recommendations for aged persons, diet components that diminish dietary calcium availability, and gastrointestinal and hormonal changes that also impact calcium availability.

The calcium RDA is 800 mg for those 51 and older; however, several researchers dispute this recommendation. Calcium requirements are determined using age and gender(12), but recommended parameters include ethnicity(2). Also, obligatory calcium losses during menopause may infer a need of 1000 mg(20). Impaired calcium absorption

TABLE III

CALCIUM INTAKE BY RACE/ETHNICITY AND AGE (60+ yo): UNITED STATES, 1988 - 91[a]

	Total Population			Non-Hispanic White		
Age	Mean	Median		Mean	Median	
Men	(mg)	(mg)	(%RDA[b])	(mg)	(mg)	(%RDA[b])
60-79y	850	710	106	871	729	109
80+y	721	638	90	742	673	93
Women						
60-79y	680	592	85	704	630	88
80+y	626	533	78	633	545	79
	Non-Hispanic Black			Mexican American		
Age	Mean	Median		Mean	Median	
Men	(mg)	(mg)	(%RDA[b])	(mg)	(mg)	(%RDA[b])
60-70y	609	498	76	788	662	98
80+y	512[c]	469[c]	64[c]	626[c]	491[c]	78[c]
Women						
60-79y	505	403	63	602	500	75
80+y	484[c]	352[c]	61[c]	595[c]	444[c]	74[c]

[a]Data based on a 24-hr dietary recall, 1 day. [b]Based on mean calcium intake. [c]Value does not meet standards of reliability or precision. Source: (1)

and diminished renal conservation can advance the requirement to 1500 mg. Since calcium needs vary during adulthood(21), the National Institutes of Health (NIH) proposed optimal calcium requirements.

Optimal calcium intake requirements promote life-long efficient use of this nutrient (see Table IV). The recommendations change for different reasons as age advances: to attain maximum peak bone mass, to maintain peak bone mass, and to minimize bone loss. The amounts of calcium for the older adult reflect the potential of poor vitamin D status, accelerated age-related bone disease, and reduced calcium absorption. Moreover, the optimal calcium intake requirements emphasize similarities of physiological calcium homeostasis among aging men and women; therefore, the optimum calcium intake recommendations are that all those over age 65 consume 1500 mg calcium daily. Hence, the needs are greater for older than younger adults; and, without effort by the individual, large portions of the population will consume insufficient amounts of calcium.

TABLE IV

OPTIMAL CALCIUM REQUIREMENT AS PROPOSED BY THE NIH

Men	Calcium (mg)
24-65 yr.	1000
Over 65 yr.	1500
Women	**Calcium (mg)**
25-50 yrs.	1000
Over 50 (postmenopausal)	1500
On estrogen	1000
No estrogen	1500
Over 65 yr.	1500

Source:(22)

Dietary Factors that Affect Calcium Requirements

The calcium requirements of a population are adversely impacted by several dietary factors. These inhibit the absorption of calcium: fiber, caffeine, phosphorus, fat, aluminum, sodium, and protein. Others, like wheat bran, phytate, and oxalate, decrease the availability of dietary calcium(23,24,25,26). One cup of coffee reduces absorption of calcium by 2 - 3 mg(27,28,29), but this effect may be offset by an additional intake of 1 - 2 tablespoons of milk(29). Though phosphorus decreases urinary calcium losses, it also increases indigenous calcium losses via the gastrointestinal tract, thus balancing the overall impact(30,31). Due to the reactions of unesterified fatty acids found in chyme and the effects of lipases, fat forms calcium soaps, diminishing the amount of available calcium. Aluminum, found in aluminum-containing antacids, increases obligatory urinary calcium losses through the binding of phosphate in the gastrointestinal tract, thus leading to reduced serum phosphate amounts and elevated urinary calcium losses. This loss can be 50 mg/day or more(32). However, the impact of these foods or medications are small relative to the effect of sodium or protein(33).

Protein and sodium alter calcium availability. Apparently, reduced intake of protein or sodium lowers calcium requirements(34), so populations with low dietary protein and sodium probably have reduced calcium needs(30,34,35,36). Consequently, a poor calcium balance can be the result of a disproportionate intake of sodium and protein. Both sodium and protein enhance calcium urinary losses whether at low or high intakes. Heaney and Recker(37), based on a study 168 female subjects between the ages of 36 to 45, predict that when dietary intakes increase from 10.8 g/day to 16.2 g/day, urinary calcium losses increase by 0.028 g/day.

For calcium, urinary losses associated with protein(38) increase glomerular filtration rates and, therefore, the filtered calcium load(39). In addition, urinary losses are associated with dietary sulfur amino acid content(40). During the catabolism of sulfur containing amino acids, such as methionine and cysteine, sulfur is oxidized to sulfate; and, as a result, hydrogen ions are formed(41). This metabolic acid diminishes the renal reabsorption of urinary calcium(42), and the sulfate cations electrostatically associate with calcium, an anion(43). Walser and Browser(44) from their study of female dogs conclude that for each millimole of sulfate excreted 0.03 millimoles of calcium are also excreted. Thus, populations with an imbalance of protein can be at risk of poor health outcomes due to calcium deficiency.

In addition to dietary factors, calcium homeostasis is impaired due to biochemical issues which are biphasic. Phase one is the first three to six years postmenopause during which rapid bone loss is related to altered amounts of the hormone estrogen(45, 46). Phase two is the subsequent postmenopausal years during which bone loss is slowed due to an altered calcium balance(45, 47). Increased and persistent losses of calcium via the kidney and intestines during this secondary phase may enhance the need for calcium intake due to hormonal changes(45). The communication of calcium-related changes among the bone, kidney, and intestines is believed to be conducted by changes in the parathyroid hormone which bathes these three organs. That is, in the presence of bone resorption leading to reduced PTH amounts, lower amounts of PTH will interact with the intestines and the kidney with the effect of reduced calcium conservation(48).

Age-related hormonal changes put segments of women at risk of a calcium deficiency called osteoporosis. Osteoporosis is a major disease of the bone defined as "a

reduction in the volume of bony tissue per unit volume of anatomical bone or a reduction in whole bone density(34)." Especially among elderly women, osteoporotic bone loss is slowed by calcium(2). Freudenheim, Johnson, and Smith(49) report that calcium supplementation reduced losses of bone mineral content from the humerus among postmenopausal women. However, Riis, Thomsen, and Christiansen(50) report that calcium supplementation did not significantly affect bone mineral content of early-postmenopausal women. In laboratory animals, osteoporosis leads to enhanced calcemia and loss of skeletal calcium to preserve obligatory fecal and urinary losses due to a calcium deficiency(34).

Calcium absorption decreases with aging just as calcium intake is reduced, which emphasizes the need for older adults to receive more of this element(45). Moreover, calcium deficiency can lead to bone changes or high blood pressure. Calcium supplementation is beneficial in the treatment and prevention of hypertension among pregnant women at risk for preclampia due to calcium deficiency(51). High blood pressure as a result of inhibition of an ATPase leading to an increase of intracellular calcium and vascular smooth muscle contraction is possibly linked to hypocalcemia. Calcium supplementation may, with essential hypertension, decrease blood pressure for some people (52, 53).

Along with affecting the vascular system, poor calcium intake during adulthood may negatively impact the bony structures. In the oral cavity, inadequate calcium intake leads to bone loss in the mandibula(54) and the alveolar bone(55). Calcium deficiency is also a risk factor for hip fracture(56, 57). Dietary calcium is negatively associated with hip fractures in a 14-year prospective study of 957 caucasian men and women between the

ages of 50 and 70 years old(57). However, in the study of 416 subjects age 65 and above(58), the risk factors for hip fracture are smoking, underweight in old age, overweight at age 20 years, weight loss, and consumption of dairy products. Thus, intakes of dietary products (milk and cheese) especially at age 20 are positively associated with a higher risk of hip fractures in old age. Perhaps the sulfur amino acid content of some dairy sources, such as cheeses, is a factor, the protein to calcium ratio being higher than in fluid milk.

Dietary Calcium Supplements

With advancing calcium demands and diminishing caloric needs, a calcium supplement may be an acceptable vehicle to increase intake among the elderly. The Food and Drug Administration (FDA) has approved several calcium supplements for use as human food ingredients (see Table V(59, 60, 61)). A supplement for this mineral can be selected by considering several attributes: the availability of the supplement, per cent of the desired nutrient, sensory impact on the food product (e.g. taste and texture), pH changes in the food product, cost of the supplementation, and effects on the availability of other dietary constituents. The bioavailability of calcium carbonate is dependent on its ability to dissolve in the stomach which is dictated by the hydrogen ion concentration of gastric secretions.

A wide range of hydrogen ion concentrations (pH) (Table VI), from 1.0 in the stomach to 8.0 in the small or large intestine, is required for proper functioning of the gastrointestinal tract. They are needed to enhance the absorption of minerals and to activate digestive enzymes. Advancing age leads to greater risk of insufficient

TABLE V

CALCIUM DIETARY SUPPLEMENTS FDA APPROVED FOR USE IN HUMAN FOODS

Calcium Supplement	Chemical Formula
Calcium carbonate	($CaC0_3$)
Calcium citrate	($Ca_3[C_6H_5O_7]_24H_20$)
Calcium glycerophoshate	($C_3H_7Ca0_6P$)
Calcium oxide	(Ca0)
Calcium pantothenate	($CaH_{16}NO_5)_2Ca$
Calcium phosphate	(Ca_3PO_4);$Ca(H_2PO_4)_2$;$CaHPO_4$
Calcium pyrophosphate	(CaP_20_7)

Compiled from several sources(59,60,61).

TABLE VI

DAILY SECRETION OF INTESTINAL JUICES

	pH
Saliva	6.0-7.0
Gastric secretion	1.0-3.5
Pancreatic secretion	8.0-8.3
Bile	7.8
Small intestine secretion	7.5-8.0
Brunner's gland secretion	8.0-8.9
Large intestinal secretion	7.5-8.0
Postprandial gastric secretion	5.8
Fasted gastric secretion for achlorhydria	$\geq$7.0
Fasted gastric secretion for healthy elderly	1.1-1.6
Fed gastric secretion for healthy elderly	3.9-5.5

Sources: (62, 64).

gastrointestinal acid production. When studying the incidence of achlorhydria (n=1951 with age groups from 10 - 99 years old), Christiansen(63) reports that its incidence was 4.7% among healthy subjects and 6.4% among patients with gastrointestinal diseases, and observes that from the fifth decade to the eighth decade the incidence rose from 1.8% to 18.5%. Russell, Berardi, Barnett, Dermentzoglou, Jarvenpaa, Schmaltz, and Dressman(64) report several significant upper gastrointestinal pH differences between the elderly and young adults. The researchers describe three types of gastric pH profiles among the elderly: a) 88% of the 79 subjects demonstrate a low fasted pH with postprandial elevation; which is similar to that of young adults; b) 5% demonstrate an elevated fasted pH with a postprandial reduction; and c) 6% demonstrate an elevated fasted pH with postprandial elevation, which they define as achlorhydria. The median meal pH of 4.9 for the elderly and 5.0 for the young, and the peak meal pH of 6.2 for the elderly and 6.6 for the young are considered similar. However, the fasted gastric acid pH of 1.3 for the elderly and 1.7 for the young are significantly different, but is concluded to be clinically insignificant. The time of 164 min. for the elderly and 64 min. for the young to reduce the postprandial gastric acid pH from 5 to 3 is significantly longer for the elderly. The elevated fasted gastric pH among 11% of the subjects is concluded to have clinical significance, because of the negative impact on the absorption of several minerals among which calcium in the form of calcium carbonate is included.

Calcium carbonate is a widely used nutrient supplement for several reasons. Its percentage of calcium is the highest (40%) on the market. It also slows the loss of compact bone among postmenopausal women(65). Among late-postmenopausal women (greater than/or equal to 6 yrs. postmenopausal), calcium carbonate is effective at

preventing decreases in bone density at the femoral neck and the radius(66). Also, calcium carbonate is inexpensive(67). Factors that appear to influence bioavailability of calcium carbonate are solubility, disintegration, and dissolution. The solubility of calcium carbonate is pH dependent with absorption increasing from 2 to 10% with a reduction in gastric pH from 6.5 to 1.0(68). For the elderly with normal fasted gastric pH, calcium carbonate as a calcium supplement can be taken in the fasted state or between meals. However, if the elderly have an elevated gastric pH in the fasted state or between meals that becomes more acid after a meal, calcium carbonate as a calcium supplement should be taken with meals for the best therapeutic effect, because normal gastric acidity enhances its absorption. Absorption of calcium from calcium carbonate is 4.2% for subjects with achlorhydria (elevated gastric pH that fall during the meal) and 22.5% for normal subjects, however, when calcium carbonate is administrated as part of a meal, the absorption rate for achlorhydric subjects is 21.2%, a value close to the normal subjects(69). However, for the elderly who demonstrate elevated fasted gastric pH that increase after a meal, calcium carbonate as a calcium supplement may be contraindicated(69, 70). Additional disadvantages to the use of calcium carbonate are that it is associated with depressed iron absorption in postmenopausal women when taken with meals and in male albino Sprague-Dawley rats(71).

In summary, in spite of these disadvantages, calcium carbonate still appears to be an effective, economical, and bioavailable calcium source for many. As stated above, calcium carbonate in powder form is 40% by weight calcium and, therefore, readily used as a calcium supplement in flour-based foods because of the ease in formulating a mixture. Calcium carbonate causes little change in sensory qualities of a food item in contrast to

several of the alternative calcium supplements, which may negatively affect the taste or texture quality of food when added at the target amount of supplementation.

Designing Foods for the Elderly

The concept of using foods as a vehicle to treat specific conditions or circumstances has long been with us. During the 1900's, iodine was added to salt of many people of several countries experiencing endemic goiter(72). Though there are many definitions for 'designer foods', the key component is adding an agent for disease prevention to a food product. But when designing a product, food technologists consider many factors. These include: safety, packaging, fortification, color, texture, flavor, fat intake, fat replacements, salt and sweetness perceptions. They must review additional factors when developing foods for the elderly: reduced food intake, the need for increased caloric density, enhancement of fluid intake(73), macronutrient replacement, carbohydrate consumption(74), changes in olfactory threshold(75), effort needed to masticate food(76), and oral health(77). For example, an older person may have difficulty consuming the volume of food which ensures adequate nutrition, or the elderly may have excess intake which promotes gain or maintenance of excess adipose tissue. Both of these circumstances can be treated by foods dense with nutrients.

There seems to be a need for nutrient dense foods that will make even small servings nutritionally adequate for the frail elderly patient. Thus, researchers in the College of Human Environmental Sciences at Oklahoma State University developed a flour blend for pasta supplemented with animal and plant proteins, and calcium. They selected pasta as a vehicle for a nutrient dense food because of its bland taste, soft texture,

neutral color, low fat and high carbohydrate contents, and as a conveyor of other nutrients such as vitamins A and C, in the tomato toppings.

The designer pasta has appealing sensory and nutritional qualities. A taste panel of active elderly people evaluated this pasta as acceptable(4). The calcium content of a 100 g cooked serving, as calcium carbonate, is similar to a glass of milk. A serving of the cooked pasta also contains 8 g of protein provided by various sources: sodium caseinate, dried egg whites, whole egg, hard red winter wheat flour, and torula yeast. The research reported in this dissertation was done to determine whether the added nutrients (protein and calcium) were adequately absorbed and utilized. However, because of concerns about the possible effect that increasing the protein in the pasta might have on calcium absorption and bone health(2) of the target market, an animal feeding study was planned prior to a human feeding study.

CHAPTER III

METHODS AND PROCEDURES

Chapter Overview

Two types of dietary comparisons were made to accomplish the objectives of this experiment. First, results from the marginal 10% protein modified-AIN diet (m-AIN) were compared to that of the marginal 10% protein pasta diet (PM). Second, results from the PM diet were compared to that of an adequate 17% protein pasta diet (PA). The pasta was prepared and incorporated into diets, which were then fed to animals and growth data recorded. From these animals, whole carcass and fecal parameters, protein parameters, and calcium and bone parameters were collected and analyzed for statistical significance. This discussion will include procedures for the preparation of pasta and diets; animal feeding and feeding sample collections; sample preparations; and determination of carcass, fecal protein, calcium, and bone parameters. In addition, experimental design and statistical analysis will be discussed.

Preparation of Pasta and Diets

The primary ingredient in the two pasta diets was the protein and calcium supplemented pasta. To prepare it, the dry ingredients were uniformly, mechanically mixed in the hopper of a La Parmigiana single screw extruder; second, the liquid was

added, incorporated into the dry ingredients, and the pasta was extruded. Third, it was boiled for 3 min. 15 sec. in distilled water and dried in a forced air oven for 13.5 hours at145°F. Fourth, the cooked and dried pasta was ground using a sample cyclone mill (UD Corporation, Boulder, CO) with a 1/23 in. screen.

After milling the pasta, it was incorporated into the diets. To the PM diet, the pasta protein was diluted to 10% with wheat starch, and then vitamins, minerals, dietary fiber, and dietary fat were added (Tables VII). The PA diet was prepared similarly to the PM diet, except that the protein was undiluted. The American Institute of Nutrition (AIN)(78) laboratory rat diet served as the basis for the m-AIN diet; but wheat starch, the

TABLE VII

EXPERIMENTAL GROUPS AND COMPOSITION OF PASTA AND EXPERIMENTAL DIETS

	Pasta g /100 g	m-AIN g /100 g	PM g /100 g	PA g /100 g
Sodium Caseinate		3.67	-	-
Calcium Caseinate		6.88	-	-
Pasta		0	43	74
Wheat starch		65.65	34.32	4.14
Sugar		10.0	10.0	10.0
Celufil		4.36	3.73	3.35
Soybean oil		4.46	3.95	3.51
Vitamin Mix		1	1	1.0
Choline		0.2	0.2	0.2
Mineral mix		3.5	3.5	3.5
DL methionine		0.3	0.3	0.3
Protein	17.0	10.1	10.0	17.0
Carbohydrate	46.8	67.4	67.2	60.3
Fiber	1.64	5.02	5.02	5.03
Fat	1.55	4.98	5.05	5.09

*Based on dietary formulation.

primary carbohydrate source in the pasta, was substituted for corn starch; and the protein was deliberately lowered to a marginal (10%) amount.

Feeding of Animal and Sample Collection

The experimental animals were 27 male weanling Sprague-Dawley rats (Sasco, Omaha, NE) from the same colony. They were housed individually in stainless steel cages with wire-mesh bottoms in a temperature and humidity-controlled animal feeding room. Prior to initiating the experiment, the rats were acclimated for 6 days while consuming a standard AIN diet. At the start of the study, the animals were 27 days old and weighed an average of 83 g. Animals were assigned to cages according to a randomized block arrangement to minimize differences due to cage placement (see Appendix B.1 for caging diagram) and were provided with the appropriate test diet and water, ad libitum, in uniform environmental conditions to promote normal development. Animals were fed a measured amount of feed daily and uneaten feed was collected and weighed. Fecal materials were collected at regular intervals, and rats were weighed weekly. According to the protocol for a protein efficiency ratio(83) (PER), the animals consumed the dietary treatments for 28 days. On the evening of the 28th day of the study, the animals were fasted, to reduced the amount of unabsorbed feed in their gastrointestinal tracts, and on the 29th day they were euthanized.

Preparation of Samples

The stages of sample preparation included: collecting feed and animal fecal samples, sedating the animals, collecting blood, removing tibias, autoclaving carcasses, drying carcass and feces, equilibrating bone samples, and digesting fecal and bone samples.

The animals were sedated with xylazine and anesthetized with ketamine hydrochloride. They were killed by a cardiac puncture, and three milliliters of cardiac blood were extracted from each animal. Cecum and large intestines were removed to reduce the amount of undigested feed in the carcasses. Both tibias were removed from each animal for bone analysis, the remaining carcass was frozen until autoclaved. During autoclaving, carcasses were heat processed at 10 psi (115°C) in aluminum foil pans, sealed with aluminum foil, for 2 hours (excluding the come-up and cool-down times). The carcasses were weighed before and after autoclaving. Each whole carcass was then ground in a food processor into a homogeneous mass and lyophilized for 10 days with before and after weights recorded to determine the moisture content.

Cardiac blood samples were centrifuged to recover the serum for the albumin analysis. All of the fecal materials were collected. However, only those from the last three weeks of the study were analyzed, being most representative of treatment residue, since during the acclimation period the animals received a basic AIN rat diet, not the experimental diets. These samples were oven-dried at 100°C for 48 hours to determine the moisture content, and then ground in a coffee bean grinder prior to additional analyses. The tibias were prepared for the bone density experiment and for the bone breaking experiment by equilibrating them for 30 days in a desiccator. In preparation for mineral analyses, the bone and fecal samples were ashed at 600°C for 5 hours, digested with 95.4% of 9% hydrochloric acid and 4.6% of 70% nitric acid, and placed in a water bath at 77°C for 12 hours. Finally, the samples were diluted with 0.1% lanthanium chloride to inhibit interference from phosphorus, aluminum, beryllium, silicon, titanium, or zirconium.

The same procedure was used for the preparation of bone samples for phosphorus analysis omitting the lanthanium chloride.

Determination of Carcass and Fecal Parameters

The carcass and fecal parameters, except those listed with the protein or calcium parameters, included feed intake, feed digestibility, carcass parameters (weight, moisture, dry matter, ether-extractable fat, and calorimetric energy), and fecal parameters (weight, ether-extractable fat, fatty acid content, and calorimetric energy). The animals were weighed weekly. Fecal and unconsumed feed samples were collected weekly. Also, samples from the last 21 days of the study, a period representative of the experimental diets, were used to determine the fecal parameters. Weekly and total feed intake were determined by subtracting uneaten and discarded feed from the total grams of feed offered to the animals. Feed digestibility was determined by calculation. Carcass dry matter and moisture were determined using lyophilization (Freeze Mobile 12, The Virtis Company, Inc.). Carcass and fecal fat content were determined using ether extraction(79). After the fat extraction procedure, fecal fatty acid concentration was determined using an extraction mixture of isopropanol, heptane, and sulfuric acid in proportions of 40:10:1 by volume(80,81,82). Next, the samples were placed in a 37.5°C water bath for 12 hours, then diluted with 60% heptane and 40% water which promoted the development of two phases. The top layer, which carried the lipids and which floated to the top of the tube without the aid of centrifugation, was removed and placed in pre-dried and weighed aluminum weigh boats. This layer was oven dried at 100°C overnight and the residue weighed. Carcass and fecal energy contents were determined using oxygen bomb

calorimetry (Parr, 1261 Calorimeter, Parr Ins. Co., Moline, IL.). Samples were formed into disk-shaped pellets, weighed, and placed inside a metal sample cup with a fuse traversing the surface of the sample. The sample was then sealed in a bomb, which was filled with oxygen. The bomb was placed in a chamber of the calorimeter, which was filled with distilled, deionized water. The calories to combust the sample were determined by the rise in temperature of the water.

Determination of Protein Parameters

Protein parameters included: carcass protein, fecal protein, serum albumin, and protein efficiency ratio. The protein content of the carcass and fecal samples were performed with the Leco FP-428 Total Combustion Nitrogen Determination System, using a factor of 6.25 to convert nitrogen to protein. Blood albumin was determined using a quantitative colorimetric method with bromocresol purple dye as the reagent (Sigma Diagnostics, St. Louis, MO, Albumin (BCP#625)) with absorbance read at 600 nm. The protein efficiency ratio was determined by dividing the total protein fed to the animals by amount of weight gain(83, 84, 85).

Determination of Calcium and Bone Parameters

Calcium and bone parameters included: several bone parameters (calcium and phosphorus content and concentrations, bone density, elongation, peak load, wall diameter at point of break, and wall thickness at point of break) and the amount of calcium and phosphorus in the tibia and feces. The calcium content of the diets, tibias, and feces was determined by flame atomic absorption spectrophotometry at a wavelength of 422.7 nm.

Tibia bone density was determined by comparing the weight of the bone in air with hydrostatic weighing by the Archimedes principle(86, 87). All of these bone breaking parameters (elongation, energy to peak, peak load, and breaking load) were determined using a universal testing machine (Instron Corp., Canton, MA with a MTS Sintech Renew upgrade, Sintech, Research Triangle Park, NC, Model No. 1122ADC) at a cross head speed of 1 mm per minute. Measurements were taken with the aid of a three point bending fixture. A tibia was supported on vertical prongs, 2 cm apart, near the epiphyseal line while force was applied to the mid-shaft of the tibia. At the cross-section of the break, the wall thickness and diameter were measured with a caliper of $\pm$0.01 mm accuracy (Scherr Tumico, Digital Caliper, Model No. EM-3002). Phosphorus was determined using single-beam spectrophotometry with molybdovanadate as the reagent composed of ammonium molybdate, ammonium metavanadate, and perchloric acid. Absorbance was read at 400 nm(88,89,90).

Experimental Design and Statistical Analyses

Two experimental designs were employed during this study: a randomized complete block design (RCBD) and a repeated measures over time (RM) in a RCBD. In the randomized block design, the diets were the treatments and the dependent variables were carcass parameters (total feed intake, feed digestibility, total weight gain, and final weight, moisture, dry matter, fat, and energy); fecal parameters(weight, fat, fatty acid, and energy); protein parameters (protein efficiency ratio, fecal protein, carcass protein, and albumin); and calcium and bone parameters (dietary calcium, fecal calcium, bone (density, dry weight, ash weight, calcium content, phosphorus content, breaking load, elongation,

energy to peak, peak load, and wall diameter and thickness)). The experimental units were the animals, and the blocks were the tiers or rows of the rack in which the rats were housed (Appendix B.1). A RM in a RCBD was also employed with five weight observations and four feed intake observations. The subunits of the RM were the weekly weight gain and the weekly feed intakes. Due to an imbalance of the data, the Type I sum of squares (SSI) was used to estimate the source of variation among the dependent variables. Type I sum of squares is a statistical procedure which adjusts the weighting of experimental variance, instead of weighing sources of variance equally. With a level of significance at $p \leq 0.05$, the data were analyzed with F-tests from the general linear model procedure(91). Differences between the means were tested using the Least Significant Difference (LSD) procedure. Where interactions were located between the means of the repeated measures portion of this study, LS means were used to detect the difference between means(92). Due to the possibility of curvature in the plots of the data, correlations for the bone and mineral parameters were determined using Spearman's correlation coefficients.

CHAPTER IV

RESULTS AND DISCUSSION

Block or Row and Side of Rack Effects

The blocks were the rows or tiers of cages. Several of the parameters had no block or row effect. These included: protein efficiency ratio, carcass protein percentage, serum albumin, bone dry weight, bone ash weight, bone calcium, total bone calcium, total bone phosphorus, bone wall thickness, total weight gain, animal final weight, carcass moisture, carcass dry matter, carcass dry matter percentage, total carcass fat, carcass ether-extracted fat, carcass energy, and fecal fatty acid percentage. Other parameters had significant row effects. Though the animals were randomly assigned to rows and the initial mean treatment weights only varied by one gram, the initial weights of the animals in rows six and seven were significantly different. Row effects for fecal protein, dietary calcium, fecal ether-extracted fat percentage, total fecal fat, and fecal energy were primarily caused by an imbalance of experimental units (animals) in the rows. Rows nine and ten contained two and one experimental units, respectively. Most of the differences for each parameter were related to a particular row which were the following: for fecal calcium, row six; bone phosphorus, rows two and six; bone breaking load, rows seven and eight; bone elongation at break, rows six and eight; feed digestibility, rows one, two, three, and seven; fecal weight, rows three and seven; and bone density and bone wall diameter, row eight. In summary, though no particular row consistently produced an

effect, several row effects were associated with bone or fecal parameters. These row effects were summaried in Appendix B.2.

As can be seen from Appendix B.1, the animal cages were positioned on both sides of the rack. Several parameters had significant side effects and one parameter had an significant side by row interaction affect. These differences may be due to an imbalance of experimental units on the side of the racks; one side contained 15 experimental units, while the side two only contained 12 experimental units. The p-values for the side, row, and side by row interactions are listed in Appendix B.3.

Marginal Protein Diets

Carcass and Fecal Parameters

Table VIII and Appendixes C and D summarize the carcass and fecal parameters. The animals on the m-AIN diet consumed less feed than the animals on the PM diet, but this difference was not significant. Also, feed digestibility ratios were similar. The animals on the m-AIN diet seemed to use their feed more efficiently, gaining the same amount of weight as their counterparts on the PM diet while consuming slightly less feed; the difference in the PERs was not significant (Table IX). Of the carcass parameters, the moisture and dry matter amounts were not significantly different between the treatments. However, the greater, though not significant, carcass fat of the rats on the PM treatments helped explain the significantly greater carcass energy content for the animals on this diet. There were no significant differences between the fecal parameters (weight, ether-extracted fat, fatty acid, total fat, and energy) of the two diet treatments. Thus, the feed,

carcass, and fecal parameters were generally similar for the animals on the m-AIN and PM diet.

In general, the fecal fatty acid composition in animals reflects the composition of dietary fat, of sloughed microvillus membrane phospholipids, and of fermented dietary fiber(93,94). Wahnon, Cogan, and Mokady(93) determined the microvillus membrane phospholipids for rats fed soybean oil (see Appendix E). Remsey and Demigne(94) determined that fermentation of cellulose by colonic bacteria led to the production of several short-chain fatty acids which were found in the feces including: acetate, propionate, and butyrate. The primary source of lipids for all treatments in this research was soy bean oil and the dietary fiber source was cellulose. Therefore, the lipid profile of the treatments feed rats' microvillous phospholipids and fecal fatty acids should be similar to those reported by Wahnon et al.(93) (see Appendix E) and reported by Remse et al.(94).

Attempts to compare the carcass composition of the animals in the study with other studies were unsuccessful because of differences in gender, diet composition, age of animals and length of the study(95, 96, 97). MacRae, Nickel, Slinger, and Neudoerffer(95) after a study on the effect on dietary carbohydrate source on carcass composition concluded that carbohydrate selection resulted in patterns of carcass composition and weight gain response. Their study also showed that a reduction in dietary fat led to a reduction in fat carcass composition. In the experimental diets of this study, wheat starch was the dietary carbohydrate source. Thus, the carcass composition of the animals on these treatments were probably impacted by the carbohydrate source as well as the dietary lipid level.

Protein Parameters

The results from the protein parameters are presented in Table IX. The protein efficiency ratios for the two marginal diets were not significantly different and indicated that the quality of the proteins from these two diets was similar. Thus, the animals gained approximately 3 g for every gram of feed eaten whether on the m-AIN diet or the PM diet. Table X shows the PERs for several foods, and those of cow's milk, meat, and fish were comparable to values of the m-AIN or PM diet.

Fecal protein was different between the rats fed the two marginal protein diets with animals on the PM diet having significantly more fecal protein. In general, there are two sources of nitrogen in feces, undigested dietary protein and endogenous protein or metabolic fecal nitrogen(98). As dietary protein(99) or dietary fiber(100) increases, fecal nitrogen excretion increases. The sources of metabolic fecal nitrogen include endogenous catabolism, endogenous secretions (that is, unabsorbed bile, intestinal and pancreatic juices), sloughed intestinal tissue(101), and plasma protein (102). Endogenous fecal nitrogen was determined by feeding a non-protein diet to animals(103). Though it may appear that the primary source of fecal nitrogen was undigested feed, studies of breads identified endogenous fecal nitrogen as the most probable primary fecal nitrogen source(104). Thus, additional research is required to determine the sources of the fecal nitrogen in this study.

The carcass protein of the rats on the m-AIN diet was significantly greater than those on the PM diet. These results may indicate differences protein digestibility. Hopkins(105) concluded that apparent protein digestibility, using total nitrogen consumed (TNC) and total fecal nitrogen (TFN), can be satisfactorily employed to compare the

$$\frac{TNC - TFN}{TNC} \times 100$$

protein digestibilities of animals with similar nitrogen intake levels. In the case of animals on the two 10% protein diets, the nitrogen intake levels were similar. Therefore, with a greater apparent protein digestibility index for the m-AIN diet (0.83) than the PM diet (0.78), differences in the fecal protein amount may reflect the digestibility of the protein sources in the two diets. A difference in digestibility may be related to the granular form of the pasta diet, since the pasta was not ground as fine as the powdered caseinate or the wheat starch composing the m-AIN diet, thus reducing the surface area of the diet particles.

Serum albumin levels of the two marginal protein diets were not significantly different and were within the normal range for male albino rats of 3.73 ± 0.53(106). Lunn and Austin(107, 108) provided rats dietary treatments based on protein to energy ratios ranging from 0.01 to 0.12 for 14 days and concluded that animals with low protein intake and excess energy consumption developed hypoalbuminemia. Apparently, the protein energy to total energy ratio (0.09) of the marginal protein diets in this study was sufficient to avoid hypoalbuminemia.

Calcium and Bone Parameters

Table XI presents the calcium and bone parameters. The mean daily calcium intake of animals on the PM diet was significantly greater by 25 mg than the m-AIN diet. Although this was statistically significant, it may be clinically insignificant in view of the size of the daily requirement. The treatment calcium concentrations were 0.68% and

0.76%, for m-AIN and PM. However, the average daily fecal calcium levels differed by little, and percent losses of calcium in the feces were not significant, 29% and 27% for the m-AIN and PM treatments. These value were lower than results from a study of four week old, male albino rats fed experimental diets of 0.5% dietary calcium and 20% protein. In those animals, fecal calcium losses ranged from 39% to 52% of dietary calcium(109). However, in this research the fecal calcium losses were not as great, but the dietary calcium amounts were higher and the protein level was only 10%.

Though there were differences in calcium intake, none of the calcium and phosphorus parameters were significantly different for the animals on the two 10% protein diets. It was difficult to contrast the bone mineral analysis of the current research with previous research because of differences in dietary protein concentration, age, and gender of the animals(110, 111, 112). The calcium to phosphorus ratio of the tibias were 2.66 and 2.45 for the m-AIN and the PM treatments. In literature, the baseline tibia calcium to phosphorus ratio for male wealing Sprague-Dawley rats was reported as 2.08(110), but changes in diet affected changes in this ratio; on higher protein diets, the calcium to phosphorus ratio of the bone was lower(110, 113).

Bone and Mineral Correlations

As shown in Appendixes F and G, several of the parameters were correlated. For the animals on the m-AIN treatment, bone density correlated with bone diameter. Bone dry weight correlated with bone (ash weight, calcium and phosphorus contents). Bone calcium content correlated with bone phosphorus content and the final weight of the animals. Bone phosphorus correlated with bone wall thickness and the final weight of the

animals. Bone breaking load and bone elongation both correlated with bone (energy to peak and peak load). Energy to peak correlated with bone peak load. Finally, bone peak load correlated with the final weight of the animals. For those animals on the PM treatment, bone density correlated with bone (elongation and diameter). Bone dry weight correlated with bone (ash weight, calcium and phosphorus contents). Bone ash weight correlated with bone (calcium and phosphorus contents). Bone calcium content correlated with bone phosphorus content. Bone breaking load correlated with the final weights of the animals.. Bone elongation correlated with bone (energy to peak and diameter). Finally, bone energy to peak correlated with bone peak load.

Pasta Diets

Carcass and Fecal Parameters

Total feed intake and feed digestibility were similar, though the weights of animals on the PA diet were significantly greater than animals on the PM diet (Table VIII and Appendixes C and D). Thus, decreasing dietary protein concentration from 17% to 10% did not affect the animals intake' of the pasta, but did decrease weight. The implication was a diet with higher protein percentages supported greater weight gain than the same feed intake amount of a lower protein concentration. Others(114, 115) found that growth of rats was retarded by low protein diets. Edoziem and Switzer(96) also concluded that rat growth increased as dietary protein concentration increased up to 25%. There were no significant differences among the other carcass parameters.

Fecal weight and fatty acid percent were similar, however, the other fecal parameters varied due to treatment. The fecal ether-extracted fat percentage, total fecal

fat, and fecal energy were significantly greater for the animals on the PA diet. Unlike the PM diet, which provided only 17% of dietary fat in the form of the pasta, the PA diet provided 30% of dietary fat as pasta. As the percent of fat provided by the pasta increased, so did fecal fat and related parameters. Both diets were only 5% total fat. However, in both treatments some of that fat was furnished from the pasta itself primarily from the egg yolk.

Protein Parameters

Of the protein parameters for animals on the pasta diets, the PER, fecal protein, and carcass protein were significantly different between the two diet treatments (Table IX). The PA treatment had a lower PER which reflected the decrease in efficiency of weight gain with increasing protein intake. Protein efficiency ratios are conducted at restricted protein amounts so that the rate limiting growth factor is protein thus maximum body weight gain is produced per unit of protein eaten(116). The greater fecal protein for the PA diet reflected the greater amount of dietary protein in the form of granulated pasta. However, increasing the protein in the pasta diet did not significantly change the serum albumin. Further, the higher protein pasta diet produced significantly larger rats, indicating that the animals were able to utilize the extra protein, particularly, since there were no differences in percent carcass fat, but the total carcass protein of animals on the PA diet was significantly more. Thus, growth, but not serum albumin, was responsive to shifts in protein concentration.

Calcium and Bone Parameters

Table XI provides a summary of the calcium and bone parameters for pasta treatments. The treatment calcium concentrations were 0.76% and 0.83%, for PM and PA. The daily calcium intake was significantly greater by 18 mg for the animals on the PA diet than those animals on the PM diet. With the increase in dietary protein, more calcium was probably excreted in the urine(40, 43). The fecal calcium levels were very similar, and the percent loss of dietary calcium in the feces for the PM and PA was 27% and 24%, respectively. These results seemed to agree with Bell, Engelmann, Sie, and Draper(117) who concluded that as dietary protein increased, fecal calcium excretion decreased. This effect, according to Moyer, Wilson, and Schedl(118), was possibly due to mucosa quantity, which was a factor of protein intake and nutritional status of the body. Bone densities did not differ, though the bone dry weight and ash weight were significantly higher indicating that the larger rats had larger bones. The bone calcium and phosphorus concentrations varied little, while the total bone calcium and phosphorus amounts were significantly greater for the rats on the PA diet, again reflecting the large bones of the larger rats. However, the bone wall parameters were similar for both sizes of rats. Bell et al.(117) also concluded that resorption of calcium from rat bone was unaffected by high protein diet, however, their animals were seven months old. The results from the present study implied that integration of calcium into the bone of growing rats may also be unaffected by dietary protein concentration. However, the maximum amount of dietary protein tested was only 17%. At even higher levels this would probably be untrue.

Several difficulties hinder the comparison of bone strength parameters with published values, primarily due to differences in the parameters determined(119, 120).

Results from the mechanical bone strength test varied slightly from treatment to treatment. While the elongation at break, wall diameter, and thickness were similar for the two treatments, the bone breaking load and peak load were significantly greater for the animals on the PA diet. These larger loads indicated that the amount of weight which could be supported by these bones prior to the breaking point was approximately 4 kg. It seems that the PA diet offered some bone-related benefits to the animals. Therefore, the extra protein did not negatively affect calcium and utilization, and possibly would have a similar affect among the elderly.

Bone and Mineral Correlations

Several of the PA parameters were correlated (Appendix H). Bone dry weight correlated with bone (ash weight and phosphorus content), and the final weight of the animals. Bone ash weight correlated with bone phosphorus content. Bone phosphorus content correlated with the final weight of the animals. Bone breaking load correlated with bone (elongation, energy to peak, and peak load), and the final weight of the animals. Bone elongation correlated with bone (energy to peak and peak load). Finally, bone energy to peak correlated with bone peak load.

Though the animals on the PA treatment were larger than the animals on the PM treatment, bone density was not correlated with weight. In humans, mean bone mineral density was greater for obese women(121), and decreases in bone mineral density correlated with fat loss in dieters(122, 123). However, in obese zucker rats which demonstrated decreased bone mass and similar bone calcium content when contrasted with

heir normal weight counterparts (124). See Appendix I for bone and mineral correlations across all the dietary treatments.

TABLE VIII

CARCASS AND FECAL PARAMETERS[a]

Parameters	Treatments			Source of Variation		
	M-AIN Mean	PM Mean	PA Mean	SE	Row p-value	Diet p-value
Total Feed Intake, g	571a	606a	601a	16	0.364	0.175
Feed Digestibility %	93.46a	93.19a	93.58a	0.2	0.062	0.381
Total Weight Gain g	184b	183b	234a	9	0.908	0.003
Animal Final Weight g	266b	266b	318a	9	0.862	0.003
Carcass Moisture %	67a	65a	66a	0.69	0.760	0.061
Carcass Dry Matter g	81a	87ba	100b	4.5	0.994	0.045
Carcass Dry Matter %	32.9a	35.3a	34.2a	0.69	0.760	0.061
Total Carcass Fat g[b]	3.18a	3.80a	4.37a	0.66	0.903	0.467
Carcass ether-extracted Fat[b]%	3.95a	4.31a	4.24a	0.6	0.728	0.892
Carcass Energy cal/g	6064a	6363b	6180ab	94	0.913	0.076
Fecal Weight g[b]	28a	31a	30a	1.1	0.046	0.287
Fecal Ether-extracted Fat[b] %	2.6a	2.6a	5.1b	0.3	0.281	0.001
Fecal Fatty Acids[b] %	0.52a	0.82ab	1.24b	0.2	0.839	0.090
Total Fecal Fat[b] g	0.92a	1.16a	1.85b	0.14	0.172	0.001
Fecal Energy cal/mg	3718a	3730a	3994b	22	0.224	0.0001

[a]$p \leq 0.05$. Means with the same letter are not significantly different. [b]Based on dry matter.

TABLE IX

PROTEIN PARAMETERS[a]

Parameters	Treatments				Source of Variation	
	M-AIN Mean	PM Mean	PA Mean	SE	Row	Diet
					p-value	
Protein Efficiency Ratio	3.12a	3.02a	2.43b	0.11	0.83	0.0006
Fecal Protein[b] g	13.83c	16.12b	25.53a	0.45	0.47	0.0001
Carcass Protein[b] g	62.17a	52.85b	59.50a	1.89	0.90	0.008
Serum Albumin g/dl	3.64a	3.74a	3.62a	0.11	0.967	0.734

[a]$p \leq 0.05$. Means with the same letter are not significantly different. [b]Based on dry matter.

TABLE X

PROTEIN EFFICIENCY RATIO

Food	PER
Human milk	4.5
Hen's egg	4.5
Cow's milk	3.0
Fish	3.0
Meat	3.0
Soy	2.5
Casein	2.5
Rice, white	1.0
Wheat grain	1.0
Bread, white	0.5
Peas and beans	0-0.5

Source: 125

TABLE XI

CALCIUM AND BONE PARAMETERS[a]

Parameters	Treatments			Source of Variation		
	M-AIN Mean	PM Mean	PA Mean	SE	Row p-value	Diet p-value
Dietary Calcium mg/day	139a	164b	182c	3.65	0.098	0.0001
Fecal Calcium mg/day	40.42a	44.32a	43.23a	1.36	0.013	0.28
Bone Density g/cm^3	1.004a	1.003a	1.002a	0.001	0.618	0.318
Bone Dry Weight mg	230a	220a	259b	7.3	0.822	0.006
Bone Ash Weight mg	7.2b	6.9b	8.0a	0.2	0.890	0.018
Bone Calcium[b] mg/g	190a	186a	199a	5.0	0.796	0.182
Total Bone Calcium[b] mg	43.6a	43.6a	48.1b	12.1	0.702	0.031
Bone Phosphorus[b] %	7.99a	8.10a	7.99a	0.07	0.014	0.447
Total Bone Phosphorus[b] mg	18.3a	17.8a	20.6b	0.51	0.479	0.004
Bone Breaking Load kg	3.1a	3.3a	3.9b	0.13	0.058	0.186
Bone Elongation @ Break mm	0.222a	0.248a	0.250a	0.013	0.038	0.231
Bone Energy to Peak N*mm	3.46a	4.26ab	5.48b	0.50	0.008	0.027
Bone Peak Load kg	2.9a	3.3a	3.9b	0.22	0.001	0.012
Bone Wall Diameter mm	2.8a	2.6a	2.9a	0.16	0.633	0.310
Bone Wall Thickness mm	0.54a	0.49a	0.54a	0.016	0.285	0.122

[a]$p \leq 0.05$. Means with the same letter are not significantly different. [b]Based on dry matter.

CHAPTER V

HYPOTHESIS TESTING, SUMMARY, AND RECOMMENDATIONS

Marginal Protein Diets

The first hypothesis (H 1.1) stated that differences in protein source would not affect the carcass and fecal parameters. The carcass energy content was greater for the animals on the PM diet. However, the other parameters were similar. These included: total feed intake; feed digestibility; total weight gain; animal final weight; carcass (moisture, dry matter, and fat); and fecal (weight, ether-extracted fat, fatty acids, and energy). Based on these results, the first hypothesis was rejected.

The second hypothesis (H 2.1) stated that differences in protein source would not affect the protein parameters of the animals on the two 10% protein diets. For the PM diet, the fecal protein was significantly greater than the m-AIN treatment, while the carcass protein was significantly lower than m-AIN treatment. The PER and the serum albumin were similar. Based on these results, the second hypothesis was rejected.

The third hypothesis (H 3.1) stated that differences in protein source would not affect the calcium and phosphorus parameters. There were no significant differences between the calcium and bone parameters: fecal calcium; and bone (density, dry weight, ash weight, calcium, phosphorus, breaking load, elongation at break, energy to

peak, peak load, wall diameter, and wall thickness). Based on this results, the researcher failed to reject the third hypothesis.

Pasta Diets

The first hypothesis (H 1.2) stated that differences in the amount of dietary protein would not affect the carcass and fecal parameters. For the PA treatment, the animal final weight; total weight gain; and fecal (ether-extracted fat, total fat, and energy) parameters were significantly greater than that of the PM treatment. Other parameters were not significantly different. These included: total feed intake; feed digestibility; carcass (moisture, dry matter, fat, and energy); and fecal (weight and fatty acids). Based on these results, the first hypothesis was rejected.

The second hypothesis (H 2.2) stated that differences in the amount of dietary protein would not affect the protein parameters of the rats. For the PA treatment, the PER was significantly lower, while the fecal protein and the carcass protein were significantly greater than for the PM treatment. The serum albumin amounts were similar. Based on these results, the second hypothesis was rejected.

The third hypothesis (H 3.2) stated that differences in the amount of dietary protein would not affect the calcium and phosphorus parameters. For the animals on the PA diet, bone (dry weight, ash weight, total calcium, total phosphorus,breaking load, and peak load) were all significantly greater than those of animals on the PM diet. Other parameters were similar: fecal calcium; and bone (density, calcium and phosphorus concentrations, break load, elongation at break, wall diameter, and wall thickness). Based on these results, the third hypothesis was rejected.

The study showed that pasta with 10% protein produced animals that grew to a similar size as animals on the m-AIN rat diet, and that supplementing the 10% protein pasta diet with calcium produced animals bones with similar calcium contents as the m-AIN diet. It also showed that changing the amount of protein in the pasta diets from 10% to 17% enhanced the growth of the animals while benefiting the calcium deposition in bones.

Recommendations

The following are the recommendations for further high-protein, high-calcium pasta studies:

(1) Evaluate the impact of the calcium source (calcium carbonate) on iron availability.

(2) Test the availability of the protein and the calcium from the pasta with the elderly and younger people with normal gastric acidity concentrations.

(3) Test the acceptability of the pasta with a large group of elderly people when incorporated into a regular meal schedule.

(4) Estimate the nutritional potential impact of this product on the diet of the target populations.

(5) Test the market feasibility of the pasta among non-elderly people such as school children.

(6) Analyze the cost of calcium and protein supplementation on the manufacturing of the product.

(7) Investigate the use of the pasta as a noodle in frozen, dried, or canned soups and dishes.

(8) Investigate the affect of high protein intake of the bone health of children, using the zucker rat as a model.

(9) Investigate the supplemented-flour blend in other flour-based foods.

REFERENCES

1. McDowell MA, Briefel RR, Alaimo K, Bischof AM, Caughman CR, Carroll MD, Loria CM, Johnson CL. *Energy and macronutrient intakes of persons ages 2 months and over in the United States: Third National Health and Nutrition Examination Survey, Phase 1, 1988-91. Advance data from vital and health statistics.* Hyattsville, Maryland: National Center for Health Statistics; 1994. no 255.

2. *NIH Consensus Development Conference on Optimal Calcium Intake,* June 6-8,1994, CME Sponsorship by National Institutes of Health, Continuing Medical Education. Bethesda, Maryland: National Institutes of Health; 1994.

3. Eaton SB, Nelson DA. Calcium in evolutionary perspective. *Am J Clin Nutr.* 1991;54:281S-287S.

4. Jafek M, Knight S, Williams, J. Acceptability of protein and calcium enriched pasta. Ninetieth Annual Meeting Southern Association of Agricultural Scientists. Tulsa, OK;1993.

5. Rudman D, Feller AG. Protein-calorie undernutrition in the nursing home. *J Amer Geriat Soc.* 1989;37:173-183.

6. Schlienger JL, Pradignac A, Grunenberger F. Nutrition of the elderly: A challenge between facts and needs. *Horm Res.* 1995;43:46-51.

7. *Nutritional Screening Manual for Professionals Caring for Older Americans.* Washington, DC: Nutrition Screening Initiative. In: Chernoff R. Meeting the nutritional needs of the elderly in the institutional setting. *Nutr Rev.* 1994;52:132-136.

8. Abbasi AA, Rudman D. Undernutrition in the nursing home: Prevalence, consequences, causes, and prevention. *Nutr Rev.* 1994;52:113-122.

9. Sempos CT, Johnson NE, Elmer PJ, Allington JK, Matthews ME. A dietary survey of 14 Wisconsin nursing homes. *J Am Diet Assoc.* 1982;81:35-40.

10. Henriken B, Cate HD. Nutrient content of food served versus food eaten in homes. *J Am Diet Assoc.* 1971;59:126-129.

11. Fauman MA. Treatment of the agitated patient with organic brain disorder. *J Amer Med Assoc.* 1978;240:380-382.
12. Food and Nutrition Board. *Recommended Dietary Allowances*, 10th ed. Washington, D.C.:National Academy Press; 1989.

13. Blumberg J. Nutrient requirements of the healthy elderly--Should there be specific RDA's?. *Nutr Rev.* 1994;52:S15-S18.

14. Munro HN. Protein nutriture and requirement in elderly people. In:Somogyi JC, Fidanza JF, eds. *Nutritional Problems of the Elderly. Bibliotheca Nutritio et Dieta, No. 33.* Basel, Karger; 1983:61-79.

15. Woteki CE, Thomas PR, eds. *Eat for Life: The Food and Nutrition Board's Guide to Reducing Your Risk of Chronic Disease.* Washington, D.C.:National Academy Press; 1992.

16. Campbell WW, Crim MC, Dallal GE, Young VR, Evans WJ. Increased protein requirements in elderly people: New data and retrospective reassessments. *Amer J Clin Nutr.* 1994;60:501-509.

17. Pannemans DLE, Halliday, D Westerterp, KR. Whole-body protein turnover in elderly men and women: Responses to two protein intakes. *Amer J Clin Nutr.* 1995;61:33-38.

18. Munro HN. Protein nutriture and requirements of the elderly. In: Munro HN. Danford DE, eds. *Human Nutrition: A Comprehensive Treatise.* Vol. 6. New York: Plenum Press; 1989:153-81.

19. Cederholm T, Jagren C, Hellstrom K. Outcome of Protein-Energy Malnutrition in Elderly Medical Patients. *Amer J Med.* 1995;98:67-74.

20. *National Health and Medical Research Council. Dietary Allowances for Use in Australia.* Australian Government Publishing Services, Canberra, 1985. Cited in: Nordin BEC. The calcium deficiency model for osteoporosis. *Nutr Rev.* 1989;47:65-72.

21. Heaney RP, Recker RR, Saville PD. Menopausal changes in calcium balance performance. *J Lab Clin Med.* 1978;92:953-963.

22. National Institute of Health, Consensus Statements NIH Consensus Development Programs, *Optimal Calcium Requirement as Proposed in NIH Consensus Development Conference Statement*: The final statement, National Institute of Health [Internet], http://text.nlm.nih.gov/.

23. Weaver CM, Heaney RP, Martin BR, Fitzsimmons ML. Human calcium absorption from wheat products. *J Nutr.* 1991;121:1769-1775.

24. Weaver CM, Heaney RP, Proulx WR, Hinders SM, Packard PT. Absorbability of calcium from common beans. *J Food Sci.* 1993;58:1401-1403.

25. Heaney RP, Weaver CM, Recker RR. Calcium absorbability from spinach. *Amer J Clin Nutr.* 1988;47:707-709.

26. Heaney RP, Weaver CM. Oxalate in vegetables: Effect on calcium absorbability. *J Bone Min Res.* 1993;8:S333.

27. Barger-Lux MJ, Heaney RP. Caffeine and the calcium economy revisited. *Osteo Intern.* 1995;5:97-102.

28. Heaney RP, Recker RR. Effects of nitrogen, phosphorus, and caffeine on calcium balance in women. *J Lab Clin Med.* 1982;99:46-55.

29. Barrett-Connor E, Chang JC, Edelstein SL. Coffee-associated osteoporosis offset by daily milk consumption. *J Amer Med Assoc.* 1994;217:280-283.

30 Heaney RD. Protein intake and calcium economy. *J Am Diet Assoc.* 1993;93:1259-1260.

31. Heaney RP, Skillman TG. Secretion and excretion of calcium by the human gastrointestinal tract. *J Lab and Clin Med.* 1964;64:29-41.

32. Spencer H, Kramer L, Norris C, Osis D. Effect of small doses of aluminum-containing antacids on calcium and phosphorus metabolism. *Amer J Clin Nutr.* 1982;36:32-40.

33. Heaney RP. Cofactors influencing the calcium requirement--other nutrients. In: *NIH Consensus Development Conferences on Optimal Calcium Intake*. Bethesda, Maryland: National Institutes of Health; 1994:71-74.

34. Nordin BEC, Morris HA. The calcium deficiency model for osteoporosis. *Nutr Rev.* 1989;47:65-72.

35. Nordin BEC, Need AG, Morris HA, Horowitz M. The nature and significance of the relationship between urinary sodium and urinary calcium in women. *J Nutr.* 1993;123:1615-1622.

36. Nordin BEC, Need AG, Morris HA, Horowitz M. Sodium, calcium and osteoporosis. In: Burckhardt P, Heaney RP, eds. *Nutritional Aspects of Osteoporosis*. New York: Raven Press; 1991;279-295.

37. Heaney RP, Recker RR. Effects of nitrogen, phosphorus, and caffeine on calcium balance in women. *J Lab Clin Med.* 1982; 99; 46-55.

38. Schuette SA, Hegsted M, Zemel MB, Linkswiler HM. Renal acid, urinary cyclic AMP, and hydroxyproline excretion as affected by level of protein sulfur amino acid, and phosphorus intake. *J Nutr*. 1981;111:2106-2116.

39. Hegsted M, Schuette SA, Zemel MB, Linkswiler HM. Urinary calcium and calcium balance in young men as affected by level of protein and phosphorus intake. *J Nutr*. 1981;111:553-562.

40. Whiting SJ, Draper HH. The role of sulfate in the calciuria of high protein diets in adult rats. *J Nutr*. 1980;110:212-222.

41. Lemann J Jr, Relman AS. The relation of sulfur metabolism to acid-base balance and electrolyte excretion: The effects of DL-methionine in normal man. *J Clin Invest.* 1959;38:2215-2223.

42. Schuette SA, Zemel MB, Linkswiler HM. Studies on the metabolism of protein-induced hypercalciuria in older men and women. *J Nutr*. 1980;110:305-315.

43. Jacob M, Smith JC, Chan JCM. Effects of metabolic acidosis on zinc and calcium metabolism in rats. *Ann Nutr Metab*. 1983;27:380-385.

44. Walser M, Browser AA Ion association. III: The effect of sulfate infusion on calcium excretion. *J Clin Invest*. 1959;38:1401-1411.

45. Gallagher JC, Riggs BL, Eisman J., Hamstra A, Arnaud SB, Duluca HF. Intestinal calcium absorption and serum vitamin D metabolites in normal subjects and osteoporotic patients: Effect of age on dietary calcium. *J Clin Invest.* 1979;64:729-736.

46. Heaney RP, Recker RR, Saville RD. Menopausal changes in bone remodeling. *J Lab Clin Med.* 1978;92:964-970.

47. Heaney RP, Recker RR, Saville PD. Menopausal changes in calcium balance performance. *J Lab Clin Med.* 1978;92:953-963.

48. Heaney RP. A unified concept of osteoporosis. *Amer J Med.* 1965;39:877-880.

49. Freudenheim JL, Johnson NE, Smith EL. Relationships between usual nutrient intake and bone-mineral content of women 35-65 years of age: Longitudinal and cross-sectional analysis. *Am J Clin Nutr*. 1986;863-76.

50. Riis R, Thomsen K, Christiansen C. Does calcium supplementation prevent postmenopausal bone loss? A double-blind, controlled clinical study. *N Eng J Med.* 1987;316: 173-177.

51. Bucher HC, Guyatt GH, Cook RJ, Hatala R, Cook DJ, Hunt D. Effect of calcium supplementation on pregnancy-induced hypertension and preeclampsia: A meta-analysis of randomized controlled trials. *JAMA*. 1996;275:1113-1117.

52. McCarron DA. Is calcium more important than sodium in the pathogenesis of essential hypertension? *Hypertension* 1985;7:607-627.

53. Resnick LM, Muller FB, Laragh JH. Calcium regulating hormones in essential hypertension: Relation to plasma renin activity and sodium metabolism. *Ann Intern Med.* 1986;108:649-654.

54. Murray EJ, Messer HH, Alamoudi N. Mandibular bone turnover during calcium deficiency in the rat. *J Dent Res.* 1982;61:1107-1109.

55. Messer HH, Goebel NK, Wilcox L. A comparison of bone loss from different skeletal sites during acute calcium deficiency in mice. *Archs Oral Biol.* 1981;26:1001-1004.

56. Cooper C, Barker DJP, Wickham C. Physical activity, muscle strength, and calcium intake in fracture of the proximal femur in Britain. *Br Med J.* 1988;297:1443-1446.

57. Holbrook TL, Barret-Connor E, Winyard DL. Dietary calcium and risk of hip fracture: 14-year prospective population study. *Lance*1988;2:1046-1049.

58. Cumming RG, Klinebery RJ. Case-control study of risk factors for hip fractures in the elderly. *Am J Epidemiol.* 1994;139:493-503.

59. Igoe RS. *Dictionary of Food Ingredients*, 2nd ed. New York: Van Nostrand Reinhold; 1989.

60. Code of Federal Regulations, Food and Drugs, 21 CFR 182.5013, April 1, 1995.

61. Weast RC, Astle J, Beyer WH, eds. *Handbook Chemistry and Physics,* 67th ed. Boca Raton, Florida: CRC Press, 1974.

62. Guyton AC, Hall JE, *Textbook of Medical Physiology*, 9th ed. Philadelphia: W.B. Saunders Company;1996.

63. Christiansen PM. The incidence of achlorhydria and hypochlorhydria in healthy subjects and patients with gastrointestinal diseases. *Scand J Gastroent.* 1968;3:497-508.

64. Russell TL, Berardi RR, Barnett JL, Dermentzoglou LS, Jarvanpaa KM, Schmaltz SR, Dressman JB. Upper gastrointestinal pH in seventy-nine healthy, elderly, North American men and women. *Pharm Res.* 1993;10; 187-196.

65. Riis B, Thomsen K, Christiansen C. Does calcium supplementation prevent postmenopausal bone loss? A double-blind, controlled clinical study. *N Eng J Med.* 1987;316:173-177.

66. Dawson-Hughes B, Ballal GE, Krall EA, Sadowski L, Sahyoun N, Tannenbaum SA. controlled trial of the effect of calcium supplementation on bone density in postmenopausal women. *N Eng J Med.* 1990;323:878-883.

67. Whiting SJ, Pluhator MM. Comparison of in vitro and in vivo tests for determination of availability of calcium from calcium carbonate tablets. *J Amer Coll Nutr.* 1992;11:553-560.

68. Ivanovich P, Fellows H, Rich C. The absorption of calcium carbonate. *Ann Intern Med.* 1967;66:917-923.

69. Recker RR, Calcium absorption and achlorhydria. *N Eng J Med.* 1985;313:70-73

70. Pak CYC, Poindexter J, Finlayson B. A model system for assessing physiochemical intestinal factors affecting calcium absorbability from the intestinal track. *J Bone Min Res.* 1989;4:119-127.

71. Dawson-Hughes B, Seligson FH, Hughes VA. Effects of calcium carbonate and hydroxyapatite on zinc and iron retention in postmenopausal women. *Am J Clin Nutr.* 1968;44:83-88.

72. Stanbury JB, Hetzel BS. *Endemic Goiter and Endemic Cretinism: Iodine Nutrition in Health and Disease.* New York, Wiley; 1980.

73. Chidester JC, Spangler AA. Fluid intake in the institutionalized elderly. *J Am Diet Assoc.* 1997;97:23-28.

74. Birge ST, Kentmann HT, Cuatrcasas P, Whedon GD. Osteoporosis, intestinal lactose deficiency and low dietary calcium intake. *N Eng J Med.* 1967; 276: 445-448.

75. Kimbrell G McA, Furchtgott E. The effect of aging on olfactory threshold. *J Gerontol.* 1963;18:364-365.

76. Gordon SR, Kelley SL, Sybyl JR, Mill M, Kramer A, Jahnigen DW. Relationship in very elderly veterans of nutritional status, self-perceived chewing ability, dental status, and social isolation. *J Am Geriatr Soc.* 1985;33:334-339.

77. Sullivan DH, Martin W, Flaxman N, Hagen JE. Oral health problems and involuntary weight loss in a population of frail elderly. *J Am Geriatr Soc.* 1993;41:725-731.

78. Reeves PG, Nielsen FH, Fahey GC. AIN-93 purified diets for laboratory rodents: Final report of the American Institute of Nutrition Ad Hoc Writing Committee on the reformulation of the AIN-76 rodent diet. *J Nutr.* 1993;123:1939-1951.

79. Official Methods of Analysis of AOAC International, 16th ed. Maryland: AOAC International, method 920.39;1995.

80. Dole VP. A relation between non-esterified fatty acids in plasma and the metabolism of glucose. *J Clin Invest.* 1956:150-154.

81. Blankenhorn DH, Ahren EH: Extraction, isolation, and identification of hydrolytic products of triglyceride digestion in man. *J Biol Chem.* 1955;212: 69-81.

82. Folch J, Lees M, Sloane-Stanely GH. A simple method for preparation of pure lipid extracts from brain. *Fed Proc.* 1954;13:209.

83. Official Methods of Analysis of AOAC International, 16th ed. Maryland: AOAC International, method 960.48;1995.

84. Derse PH. Evaluation of protein quality (biological method). *J AOAC.* 1960;43:38-41.

85. Derse PH. Evaluation of protein quality (biological method). *J AOAC.* 1965;48:849-850.

86. Kalu DN, Liu CC, Harlin RR, Holiss BW. The aged rat model of ovarian deficiency bone loss. *Endocrinology* 1994;124:7-16.

87. Seaborn CD, Nielsen FH. High dietary aluminum affects the response of rats to silicon deprivation. *Biol Trace Elem Res.* 1994;41:295 -304.

88. Official Methods of Analysis of AOAC International, 16th ed. Maryland: AOAC International, method 965.17;1995.

89. Heckman M. Collaborative study of the photometric molybdovanadate method of phosphorus. *J AOAC.* 1965;48;654-657.

90. Hoover WL. Comparison of wet ash and dry as methods for the digestion of mineral-mix feeds. *J AOAC.* 1976;59:937-938.

91. SAS Software, Raleigh, SAS Institute, Raleigh, NC;1985.

92. Torrie JH, Steel RGD. *Principles and Procedures of Statistics.* 2nd ed. New York: McGraw-Hill Book Co.;1980.

93. Wahnon R, Cogan U, Mokady S. Dietary fish oil modulates the alkaline phosphatase activity and not the fluidity of rat intestinal microvillus membrane. *J Nutr*. 1992;122:1077-1084.

94. Remsey C, Demigne C. Partition and absorption of volatile fatty acids in the alimentary canal of the rat. *Ann Rech Vet*. 1976;7:39-55.

95. MacRae AR, Nickel I, Slinger SJ, Neudoerffer TS. Carcass composition and weight gain response of rats fed various carbohydrates at two dietary levels of carbohydrate and protein. *Nutr Metabol*. 1974;17:47-54.

96. Edozien JC, Switzer BR. Influence of diet on growth in the rat. *J Nutr*. 1978;108:282-290.

97. Jen KLC. Effects of diet composition on food intake and carcass composition in rats. *Physiol Behav*. 1988:42;551-556.

98. Hegsted DM. Protein Requirements. In: Munro HN, Allison JB, eds., *Mammalian Protein Metabolism, vol 2*. NY:Academic Press; 1964:135-171

99. Harmon BG, Becker DE, Jensen AH, Baker DH. Influence of microbiotia on metabolic fecal nitrogen in rats. *J Nutr*. 1968;96:391-396.

100. Shah N, Atallah MT, MaHoney RR, Pellet PL. Effect of dietary fiber components on fecal nitrogen excretion and protein utilization in growing rats. *J Nutr*. 1982;112:658-666.

101. Scheider BH. The relationship of metabolic nitrogen of the feces to body weight and to food intake for rats. *Biochem J*. 1934;28:360.

102. Matthews DM. Intestinal absorption of peptides. *Physiol Rev*. 1975;55:587-608.

103. Pellet PL, Young VR. *Nutritional Evaluation of Protein Foods, WHTR-3/UNUP-129*. Tokyo, Japan: The United Nations University; 1980.

104. McCance RA, Walsham CM. The digestibility and absorption of the calories, protein, purines, fat and calcium and wholemeal wheaten bread. *Br J Nutr*. 1948;26-40.

105. Hopkins DT. Effects of variation in protein digestibility. In: *Protein Quality In Humans: Assessment and in Vitro Estimation*. Connecticut:The AVI Publishing Co.; 1981:169-193.

106. Mitruka BM, Rawnsley HM. *Clinical Biochemical and Hematological References Values in Normal Experimental Animal*. New York: Masson Pub.; 1977.

107. Lunn PG, Austin S. Excess energy intake promotes the development of hypoalbuminaemia in rats fed on low-protein diets. *Br J Nutr.* 1983;49;9-15.

108. Lunn PG, Austin, S. Dietary manipulation of plasma albumin concentration. *J Nutr.* 1983;113:1791-1802.

109. Shenolikar IS, Rao BS. Influence of dietary protein on calcium metabolism in young rats. *Ind Jour Med Res.* 1968;56:1412-1421.

110. Peterson CA, Eurell JC, Erdman JW. Bone composition and histology of young growing rats fed diets of varied calcium bioavailability: Spinach, nonfat dry milk, or calcium carbonate added to casein. *J Nutr.* 1992;122:137-144.

111. Persson P, Gagnemo-Persson R, Hakanson R. The effect of high or low dietary calcium on bone and calcium homestasis in young male rats. *Calcif Tissue Int* 1993;52:460-464.

112. Peterson, CA, Eurell, JC, Erdman, JW. Alterations in calcium intake on peak bone mass in the female rat. *J Bone Miner Res.* 1995;10:81-95.

113. Kawashima T, Hideo Y. The effect of high calcium and/or high protein diet on bone growth in growing rats kept at high ambient temperature. *J Nutr Sci Vitaminol.* 1988;34:47-54.

114. Anthony LE, Edozien JC. Experimental protein and energy deficiencies in the rat. *J Nutr.* 1975;105;631-648.

115. Mercer LP, Watson DF, Ramlet JS. Control of food intake in the rat by dietary protein concentration. *J Nutr.* 1981;111:1117-1123.

116. Osborn TB, Mendel LB, Ferry EL. A method of expressing numberically the growth-promoting value of proteins. *J Biol Chem.* 1919:37;223-229.

117. Bell RR, Engelmann DT, Sie T, Draper HH. Effect of a high protein intake on calcium metabolism in the rat. *J Nutr.* 1975;105:475-483.

118. Moyer GL, Wilson HD, Schedl HP. Effect of dietary protein on body weight, mucosal growth, and calcium absorption in the rat. *Am J Digestive Dis.* 1978;23:545-549.

119. Weir JB, Bell GH, Chambers JW. The strength and elasticity of bone in rats on a rachitogenic diet. *J Bone Jt Surg.* 1949;31B:444-451.

120. Bell GH, Curthberson DP, Orr J. Strength and size of bone in relation to calcium intake. *J Physiol.* 1941;100:299-317.

121. Albala C, Yanez M, Devoto E, Sostin C, Zeballos L, Santos JL. Obesity as a protective factor for postmenopausal osteoporosis. *Int J Obes.* 1996;20:1027-1032.

122. Prichard JE, Nowson CA, Wark JD. Bone loss accompanying diet-induced or exercise-induced weight loss: a randomised controlled study. *Int J Obes.* 1996;20:513-520.

123. Jensen LB, Quaade F, Sorensen OH. Bone loss accompanying voluntary weight loss in obese humans. *J Bone Miner Res.* 1994;9:459-463.

124. Foldes J, Shih M, Levy J. Bone structure and calcium metabolism in obese zucker rats. *Int J Obes.* 1992;16:95-102.

125. Bender AE. Nutritional Value of Proteins and Its Assessment. In: Fox, PF, Condon , JJ ed. *Food Proteins.* London: Applied Science Publishers; 1982:121-131.

APPENDIXES

APPENDIX A

REVISION OF RESEARCH OBJECTIVES

The following were the initial objectives and hypotheses of this study which involved 40 animals and four experimental diets:

A. Two marginal protein (10%) diets, one based on the AIN rat diet and one that derived its protein from the diluted supplemented pasta.

B. Two 17% (adequate) protein diets, one that was the based on the AIN rat diet and the other drew its protein from the modified pasta.

All diets were adjusted so that they were similar in calcium amount (as $CaCO_3$) and other nutrients, except protein. However, these objectives were altered because, upon analysis of the diets, the supposed 17% protein modified AIN diet was actually only 12% protein. This necessitated removal of the 10 animals on this treatment from this study.

Based on the above research objectives, the following are the initial hypothesis for this research project:

1. To determine whether the protein in the supplemented pasta is efficiently absorbed and utilized.

H1: There will be no differences in mean intakes, weight gain, or protein efficiency ratios of the rats the four test diets due to treatments.

H2: There will be no differences in the animals' carcass composition, serum albumin, or diet composition (percentage fat, protein, moisture, and energy) due to protein level in the diet.

2. To determine if the protein level in the diet would interfere with the absorption and assimilation of calcium.

H3: There will be no differences in the breaking strength of the animal bones due to the protein content of the diets.

H4: There will be no difference in calcium and phosphorus contents of the bones due to protein content in the diets.

H5: There will be no difference in fecal calcium, protein, or saponified fats due to protein content in the diet.

3. To determine whether total body composition is affected by protein level in the diet.

H6: There will be no difference in carcass composition due to the amount of protein in the diets.

APPENDIX B.1

ARRANGEMENT OF EXPERIMENTAL
UNITS IN CAGES, COLUMNS, ROWS,
AND SIDES OF RACK

Rack Side 1

	Column #				
	1	2	3	4	
Row or Tier			Treatments		
1		22 PM		23 PA	24 M-AIN
2	25 M-AIN	26 PA		27 PM	
3	29 PM			31 M-AIN	32 PA
4	33 M-AIN	34 PA			36 PM
5	37 M-AIN	38 PA		39 PM	

Rack Side 2

	Column #				
	1	2	3	4	
Row or Tier			Treatments		
6	1 M-AIN	2 PA			4 PM
7	5 M AIN	6 PA			8 PM
8	9 PM	10 M-AIN			12 PA
9		X 14 M-AIN		15 PA	16 PM
10	X 17 PM	X 18 PA		19 AIN-M	

X: Missing experimental units. Rows going across represents a block in the experiment design.

APPENDIX B.2

ARRANGEMENT OF EXPERIMENTAL UNITS IN CAGES, COLUMNS, ROWS, AND SIDES OF RACK WITH PARAMETERS WITH SIGNIFICANT ROW EFFECTS SUMMARIED

Rack Side 1

	Column #			
	1	2	3	4
Row or Tier	Treatments			
1		22 PM	23 PA	24 M-AIN
Parameters with significant row effects in Row 1: Feed Digestibility.				
2	25 M-AIN	26 PA	27 PM	
Parameters with significant row effects in Row 2: Bone Phosphorus; and Feed Digestibility.				
3	29 PM		31 M-AIN	32 PA
Parameters with significant row effects in Row 3: Feed Digestibility; and Fecal Weight.				
4	33 M-AIN	34 PA		36 PM
Parameters with significant row effects in Row 4: None.				
5	37 M-AIN	38 PA	39 PM	
Parameters with significant row effects in Row 5: None.				

Rack Side 2

	Column #			
	1	2	3	4
Row or Tier	Treatments			
6	1 M-AIN	2 PA		4 PM
Parameters with significant row effects in Row 6: Initial Weights; Fecal Calcium; Bone Phosphorus; and Bone Elongation at Break.				
7	5 M AIN	6 PA		8 PM
Parameters with significant row effects in Row 7: Initial Weights; Bone Breaking Load; Feed Digestibility; and Fecal Weight.				
8	9 PM	10 M-AIN		12 PA
Parameters with significant row effects in Row 8: Bone Breaking Load; Bone Elongation at Break; Bone Density; and Bone Wall Diameter.				
9		X 14 M-AIN	15 PA	16 PM
Parameters with significant row effects in Row 9: Fecal Protein; Dietary Calcium; Fecal Calcium; Bone elongation at break; Fecal Weight; Fecal Fat; and Fecal Energy.				
10	X 17 PM	X 18 PA	19 AIN-M	
Parameters with significant row effects in Row 10: Fecal Protein; Dietary Calcium; Bone Phosphorus; Bone elongation at break; and Bone peak load.				

X: Missing experimental units. Rows going across represents a block in the experiment design.

APPENDIX B.3

SIDE, ROW, AND SIDE BY ROW INTERACTION P-VALUES FOR THE CARCASS, FECAL, PROTEIN, CALCIUM, AND BONE PARAMETERS

Parameters	Side	Row	Side*Row
		p-value	
Total Feed Intake	0.8507	0.5907	0.4425
Feed Digestibility	0.0023	0.1034	0.3889
Total Weight Gain	0.6293	0.8790	0.9699
Animal Final Weight	0.7390	0.8388	0.9183
Carcass Moisture	0.1452	0.8771	0.9670
Carcass Dry Matter	0.2052	0.7876	0.9502
Total Carcass Fat	0.9312	0.8036	0.6798
Carcass ether-extracted fat	0.9775	0.6635	0.4024
Carcass Energy	0.5026	0.7041	0.9827
Fecal Weight	0.0139	0.7565	0.1426
Fecal Ether-extracted Fat	0.7940	0.3660	0.8313
Fecal Fatty Acids	0.4525	0.7043	0.9503
Total Fecal Fat	0.7765	0.2854	0.7680
Fecal Energy	0.9658	0.9378	0.8176
Protein Efficiency Ratio	0.9240	0.9958	0.7418
Fecal Protein	0.6518	0.9239	0.9452
Carcass Protein	0.8292	0.8397	0.9517
Serum Albumin	0.2929	0.5547	0.5288
Dietary Calcium	0.9803	0.6091	0.6289
Fecal Calcium	0.0137	0.2981	0.0271
Bone Density	0.3639	0.3067	0.9551
Bone Dry Weight	0.5951	0.9791	0.7740
Bone Ash Weight	0.8961	0.9830	0.7855
Bone Calcium	0.7098	0.7386	0.5186
Total Bone Calcium	0.6063	0.7626	0.7202
Bone Phosphorus	0.0560	0.0046	0.2914
Total Bone Phosphorus	0.9128	0.5605	0.7608
Bone Breaking Load	0.0368	0.4945	0.0639
Bone Elongation@ Break	0.0040	0.1128	0.2104
Bone Energy to Peak	0.00063	0.5432	0.2284
Bone Peak Load	0.0034	0.7445	0.1981
Bone Wall Diameter	0.1785	0.7901	0.6762
Bone Wall Thickness	0.6195	0.2587	0.3930

APPENDIX C

DIETS BY WEEKLY WEIGHT GAINS
(N=9)

Marginal Diets	Week of Study	Means
m-AIN	0	82[a]
PM	0	83[a]
m-AIN	1	51[b,f,g,h,i]
PM	1	47[c,g,j,k,l]
m-AIN	2	47[d,h,j,m]
PM	2	38[p]
m-AIN	3	47[e,i,k,m,n]
PM	3	53[b,c,d,e]
m-AIN	4	38[o,p]
PM	4	43[l,n,o]
Pasta Diets	**Week of Study**	**Means**
PM	0	83[a]
PA	0	83[a]
PM	1	49[i,j]
PA	1	67[b]
PM	2	38[k]
PA	2	53[d,f,h,i]
PM	3	53[e,g,h,]
PA	3	60[b,c,d,e]
PM	4	43[j,k]
PA	4	54[c,f,g]

$p \leq 0.05$ Means with the same letter are not significantly different. Means are in descending order.

APPENDIX D

DIETS BY WEEKLY FEED INTAKES
(N=9)

Marginal Diets	Week of Study	Mean
PM	3	178[a]
m-AIN	3	168[a,b]
PM	4	168[b]
m-AIN	4	146
m-AIN	2	136[d,c]
PM	2	133[c,e,f]
PM	1	127[d,e,f]
m-AIN	1	121[f,g]
Pasta Diets	**Week of Study**	**Mean**
PM	3	178[a,b]
PA	3	177[a]
PA	4	168[b,c]
PM	4	168[c]
PA	2	136[d,e]
PM	2	133[d,f]
PM	1	127[e,f,g]
PA	1	121[g]

$p \leq 0.05$ Means with the same letter are not significantly different. Means are descending order.

APPENDIX E

FATTY ACID COMPOSITION OF SOYBEAN OIL AND MICROVILLUS MEMBRANE PHOSPHOLIPIDS OF RATS FED SOYBEAN OIL

Fatty Acid	Trivial Name[b]	Soybean Oil g /100 g fatty acid	Microvillus Membrane[c] g /100 g fatty acid Mean ± SEM
12:0[a]	Lauric	0.1	1.8 ± 0.7
14:0	Myristic	4.5	3.4 ± 0.4
14:1		-	1.0 ± 0.2
16:0	Palmitic	11.6	25.7 ± 1.8
16:1		-	1.6 ± 0.8
18:0	Stearic	2.5	28.3 ± 2.3
18:1		21.1	13.6 ± 0.9
18:2(*n*-6)	Linoleic	52.4	15.6 ± 1.1
18:3	γ-Linolenic	7.1	-
20:1		-	0.9 ± 0.2
20:4(*n*-6)	Arachidonic	-	1.3 ± 0.8
20:5(*n*-3)		-	0.5 ± 0.1
22:1		-	1.2 ± 0.2
22:5(*n*-3)		-	0.9 ± 0.3
22:6(*n*-3)		-	1.6 ± 0.4

[a]The number before colon is the number of carbon atoms. The number after the colon is the number of double bonds. *n* represents the carbon number when counting is started from the methyl end of the fatty acid where the first double bond is located. [b]Source: Chow CK, ed. *Fatty Acids in Foods and Their Health Implications.* New York: Marcel Dekker, Inc; 1995. [c]Source: Wahnon R, Cogan U, Mokady S. Dietary fish oil modulates the alkaline phosphatase activity and not fluidity of rat intestinal microvillus membrane. *J Nutr.* 1992;122:1077-1084.

APPENDIX F

SPEARMAN'S CORRELATION COEFFICIENT OF FINAL WEIGHT
AND BONE PARAMETERS AND FINAL WEIGHT FOR THE M-AIN DIET
(N=9)

	Bone Dry Weight	Bone Ash Weight	Bone Ca^{++}	Bone P	Breaking Load	Bone Elongation	Energy to Peak	Bone Peak Load	Bone Diameter	Bone Wall Thickness	Final Weight
Bone Density	0.45 NS	0.15 NS	0.18 NS	0.23 NS	0.017 NS	-0.08 NS	-0.08 NS	-0.02 NS	0.76 0.02	-0.05 NS	0.32 NS
Bone Dry Weight		0.78 0.01	0.82 0.01	0.82 0.01	-0.20 NS	-0.08 NS	-0.07 NS	-0.13 NS	0.32 NS	0.55 NS	0.63 NS
Bone Ash Weight			0.38 NS	0.62 NS	-0.62 NS	-0.15 NS	-0.35 NS	-0.52 NS	0.12 NS	0.47 NS	0.20 NS
Bone Ca^{++}				0.80 0.01	0.22 NS	0.03 NS	0.26 NS	0.26 NS	0.17 NS	0.63 NS	0.80 0.01
Bone P					-0.22 NS	-0.23 NS	-0.12 NS	-0.17 NS	0.37 NS	0.87 0.01	0.68 0.04
Breaking Load						0.58 NS	0.83 0.005	0.93 0.0002	0.08 NS	-0.22 NS	0.47 NS
Bone Elongation							0.88 0.002	0.76 0.01	-0.07 NS	-0.06 NS	0.30 NS
Energy to Peak								0.97 0.0001	0.03 NS	0.03 NS	0.45 NS
Peak Load									0.07 NS	-0.07 NS	0.45 0.22
Bone Diameter										0.30 NS	0.38 NS
Bone Wall Thickness											0.53 NS

APPENDIX G

SPEARMAN'S CORRELATION COEFFICIENT OF BONE PARAMETERS AND FINAL WEIGHT FOR THE PM DIET (N=9)[a]

	Bone Dry Weight	Bone Ash Weight	Bone Ca^{++}	Bone P	Breaking Load	Bone Elongation	Energy to Peak	Bone Peak Load	Bone Diameter	Bone Wall Thickness	Final Weight
Bone Density	0.45 NS	0.35 NS	0.25 NS	0.55 NS	-0.27 NS	-072 0.03	-0.40 NS	-0.32 NS	0.77 0.01	0.36 NS	0.58 NS
Bone Dry Weight		0.97 0.0001	0.83 0.01	0.93 0.0002	0.52 NS	-0.27 NS	0.20 NS	0.43 NS	0.51 NS	-0.05 NS	-0.08 NS
Bone Ash Weight			0.78 0.01	0.90 0.001	0.63 NS	-0.22 NS	0.27 NS	0.52 NS	0.46 NS	0.00 NS	-0.25 NS
Bone Ca^{++}				0.87 0.002	0.62 NS	-0.03 NS	0.33 NS	0.53 NS	0.32 NS	0.36 NS	-0.18 NS
Bone P					0.52 NS	-0.25 NS	0.25 NS	0.47 NS	0.56 NS	0.28 NS	-0.15 NS
Breaking Load						0.28 NS	0.55 NS	0.65 NS	-0.13 NS	0.29 NS	-0.73 0.02
Bone Elongation							0.83 0.005	0.65 NS	-0.74 0.02	-0.12 NS	-0.50 NS
Energy to Peak								0.93 0.0002	-0.53 NS	0.05 NS	-0.57 NS
Bone Peak Load									-0.33 NS	0.12 NS	-0.63 NS
Bone Diameter										0.07 NS	0.26 NS
Bone Wall Thickness											-0.05 NS

[a]For Bone Wall Thickness n=8.

APPENDIX H

SPEARMAN'S CORRELATION COEFFICIENT OF BONE PARAMETERS AND FINAL WEIGHT FOR THE PA DIET (N=9)

	Bone Dry Weight	Bone Ash Weight	Bone Ca^{++}	Bone P	Breaking Load	Bone Elongation	Energy to Peak	Bone Peak Load	Bone Diameter	Bone Wall Thickness	Final Weight
Bone Density	-.0.63 NS	-0.52 NS	-0.80 NS	-0.50 NS	-0.42 NS	-0.53 NS	-0.52 NS	-0.60 NS	-0.36 NS	-0.32 NS	-0.27 NS
Bone Dry Weight		0.88 0.001	0.58 NS	0.88 0.002	0.57 NS	0.47 NS	0.50 NS	0.45 NS	0.05 NS	0.42 NS	0.83 0.005
Bone Ash Weight			0.32 NS	0.80 0.01	0.30 NS	0.18 NS	0.20 NS	0.20 NS	0.18 NS	0.47 NS	0.65 NS
Bone Ca^{++}				0.52 NS	0.28 NS	0.33 NS	0.37 NS	0.45 NS	0.38 NS	0.33 NS	0.37 NS
Bone P					0.30 NS	0.32 NS	0.35 NS	0.40 NS	0.05 NS	0.42 NS	0.68 0.04
Breaking Load						0.90 0.001	0.88 0.002	0.78 0.01	-0.10 NS	-0.25 NS	0.67 0.05
Bone Elongation							0.98 0.0001	0.92 0.0005	-0.07 NS	-0.35 NS	0.40 NS
Energy to Peak								0.87 0.0025	-0.05 NS	-0.37 NS	0.42 NS
Bone Peak Load									0.17 NS	-0.33 NS	0.35 NS
Bone Diameter										-0.25 NS	-0.17 NS
Bone Wall Thickness											0.32 NS

APPENDIX I

SPEARMAN'S CORRELATION COEFFICIENT OF BONE PARAMETERS AND FINAL WEIGHT FOR ALL TREATMENTS (n=27)[a]

	Bone Dry Weight	Bone Ash Weight	Bone Ca^{++}	Bone P	Breaking Load	Bone Elongation	Energy to Peak	Bone Peak Load	Bone Diameter	Bone Wall Thickness	Final Weight
Bone Density	-0.07 NS	-0.11 NS	-0.16 NS	0.01 NS	-0.21 NS	-0.39 0.04	-0.33 NS	-0.34 NS	0.22 NS	0.08 NS	0.02 NS
Bone Dry Weight		0.94 0.0001	0.92 0.0001	0.95 0.0001	0.32 NS	0.002 NS	0.26 NS	0.33 NS	0.46 0.01	0.51 0.0081	0.65 0.0002
Bone Ash Weight			0.78 0.0001	0.87 0.0001	0.23 NS	-0.10 NS	0.14 NS	0.22 NS	0.48 0.01	0.44 0.02	0.50 0.01
Bone Ca^{++}				0.89 0.0001	0.37 0.05	0.10 NS	0.34 NS	0.48 0.02	0.43 0.02	0.54 0.005	0.69 0.0001
Bone P					0.30 NS	-0.03 NS	0.27 NS	0.33 NS	0.46 0.01	0.60 0.001	0.68 0.0001
Breaking Load						0.61 0.001	0.78 0.0001	0.81 0.0001	0.05 NS	-0.02 NS	0.28 NS
Bone Elongation							0.92 0.0001	0.80 0.0001	-0.29 NS	-0.29 NS	0.11 NS
Energy to Peak								0.93 0.0001	-0.11 NS	-0.17 NS	0.31 NS
Bone Peak Load									0.04 NS	-0.06 NS	0.32 0.10
Bone Diameter										0.18 NS	0.28 NS
Bone Wall Thickness											0.37 NS

[a]For Bone Wall Thickness n=26.

VITA

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Doctor of Science

Dissertation: NUTRITIONAL AVAILABILITY FROM A HIGH-CALCIUM, HIGH PROTEIN PASTA

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